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ABSTRACTS OF PAPERS PRESENTED AT THE 1961 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

LAFAYETTE, INDIANA, AUGUST 28 TO AUGUST 30, 1961

ABRAHAMSON, S., University of Wisconsin, Madison, Wis.: *Possible repair of X-ray induced mutation in Drosophila melanogaster.*—DR. NEWTON MORTON'S observation that irradiated sperm produced a shift in sex ratio significantly larger than expected has resulted in our doing several large-scale experiments to determine underlying mechanisms. Males (*v dy g*) receiving 2700r and corresponding controls were mated either to *sc*^{81} *B InS w*^{a} *sc*^{8} (Basc = M-5) females or to *y f* reversed acrocentric females needing the Y chromosome for survival. Mating (1) yields the sex-linked recessive lethal rate; the second gives the induced sex ratio shift, found to be two or more times greater than expected from the lethal frequency. Dominant lethality, detrimental but nonlethal mutations and mosaicism were considered as possible causes for the discrepancy.—In the sex ratio scheme, loss of X or Y chromosome results in zygotic death; possible unequal sex chromosome dominant lethality should not be great enough to produce the observed shift.—Mating the F_1 patroclinous males to compound X females produced the control sex ratio rate, indicating that detrimental mutations were not the cause.—Mating about 1000 F_2 females from nonlethal Basc cultures produced an F_3 lethal rate corresponding to the control. Furthermore no lethals reverted. Therefore, lethal gonadal mosaicism was not operating to any appreciable extent.—If these factors play little role in producing the observed phenomenon, it seems likely that the sex ratio shift depends almost exclusively on sex-linked recessive lethality.—As a possible hypothesis, some induced mutations may be metastable. In the male zygote considerably fewer mutants might be repaired than in the female zygote. On this hypothesis the agent responsible for repair may be the unirradiated X. Alternatively somatic mosaicism without gonadal mosaicism resulting from early segregation in a two or more stranded chromosome structure remains a possibility. (This investigation was supported by a research grant, RG7666, from the National Institutes of Health, Public Health Service.)

AHLOOWALIA, B. S., and R. SNOW, University of California, Davis, California: *Variable chromosomal expression in the karyotype of Clarkia amoena (Lehmann) Nelson and Macbride.*—The expression of chromosome morphology in the somatic karyotype, although generally considered fairly constant, may show wide variations in the same preparation from cells of the same tissue. The individual chromosomes in the root tip karyotype of a strain of *C. amoena* showed variable expression of differentially staining regions, the absence or presence of a lightly staining region leading to variable morphology of the homologous pair in the same cell. To test whether such variable expression was the result of differential contraction of these regions, the root tips were treated before fixation with 0.025% solution of colchicine at 21°C for times from 15 to 120 minutes. A statistical comparison of the diploid genome length in the treated and control series showed that contraction of the chromosomes was first evident after 75 minutes of pretreatment, and that there was no further contraction up to 120 minutes. The number of lightly staining regions per cell was found to be constant irrespective of the degree of chromosome contraction. The ratio of the length of a particular chromosome to that of the haploid genome was found to be constant. The variation in the number of regions of the individual chromosome may be related to the asynchronous replication of DNA in the chromosome as reported by other authors.

ALBRECHT, A. M., R. H. VOGEL, and H. J. VOGEL, Institute of Microbiology, Rutgers University, New Brunswick, N. J.: *Enzyme repression in arginine pathway: common genetic control and individual physiological response.*—Acetylornithine delta-transaminase (ALBRECHT and VOGEL, Federation Proc. 19:2, 1960) and acetylornithinase are repressible by arginine in strain

W, but not strain B, of *Escherichia coli*. However, the repression of the two enzymes in W is non-co-ordinate. In arginine-requiring strain 39A-23R3, derived from W, mixed supplements of arginine and acetylornithine can support diphasic growth, with preferential utilization of arginine (first phase) and subsequent utilization of acetylornithine (second phase), at lowered exponential growth rate. During second-phase growth, derepression of the transaminase and acetylornithinase results. The latter enzyme shows "pace-setting" (VOGEL, Biochem. Biophys. Research Comm. 3:373, 1960); the former does not. A strain B mutant, analogous to 39A-23R3, after preliminary cultivation on arginine, grows at *undiminished* rate on acetylornithine; mixed supplements give monophasic exponential growth. These findings support earlier indications that acetylornithine permease is repressible in W, but not in B. In certain recombinants resulting from crosses between B as recipient and the repressible K-12 strain of *E. coli* as donor, and kindly furnished by DR. L. GORINI, the transaminase and acetylornithinase are repressible. Thus, the same genetic determinant appears to control the repressibility of the two enzymes and the permease; nevertheless, as shown by non-co-ordination and differential pace-setting, the response of the corresponding synthesizing systems to arginine is given *individually*. (Aided by Damon Runyon Fund, Office of Naval Research, and U. S. Public Health Service.)

ALEXANDER, MARY L., Section of Radiation Biology, The University of Texas, M. D. Anderson Hospital, Houston, Texas, and M.R.C. Radiopathological Unit, Hammersmith Hospital, London: *Genetic recovery mechanisms and fast neutron treatment of mature sperm treated in males and fertilized females of D. melanogaster.*—Fast neutrons of an average of 2 Mev energies were utilized to test for increases in sensitivity in mature sperm after female insemination. Mature males and inseminated females were treated with 900 rads of neutrons. Males were mated to Muller-5 females or *bw st* females and the first and second day's tested separately. Sex-linked lethals were scored by the Muller-5 method and translocations tested with *bw st*.—The results for sex-linked lethals induced in sperm treated in inseminated females were 5.5% (5/90) for the first day and 8.3% (51/611) for the second. For treated males, the percentages of lethals were: first day 5.4% (40/735) and the second day 3.5% (17/488).—For translocations in the female test, 9.8% (47/479) were obtained for the first day and 9.5% (59/623) for the second. For treated males, 5.2% (33/629) were found for the first day and 7.8% (43/552) for the second day. By treating males with 1500 rads of neutrons, translocation values of 14.1% (30/212) were observed for the first day and 11.8% (22/211) for the second day. Chi-square values were not significant for the first and second day comparisons.—The absence of significant or consistent changes in genetic damage in the second day as compared to the first indicates the absence of recovery mechanisms after neutron treatment. Combined values for both days were significantly higher in the female than in the male tests. This increase in genetic damage after sperm insemination shows greater chromosome sensitivity to breakage since recovery mechanisms were not observed and will not explain the increase. (Supported by a Public Health Service—M.C.I. Special Fellowship, Institute of Animal Genetics, Edinburgh, Scotland and Contract AT[14–1]–1828 with the United States Atomic Energy Commission and the University of Texas M.D. Anderson Hospital, Houston, Texas.)

ALLEN, ARCHIE C., and E. B. SPIESS, University of Pittsburgh, Pittsburgh, Pa.: *Effects of recombination on viability in second and third chromosomes of Drosophila melanogaster.*—One hundred second and third chromosomes were tested simultaneously for viability in homozygous condition after extraction from a laboratory population of *D. melanogaster* which had been maintained for more than 170 generations at 25°C (WALLACE's Oregon-R control #3, 1949, population). Ten strains with "normal viability" for both chromosomes were chosen for crossing and were kept in balanced condition (*Cy L, Ubx* balancers). Each strain was crossed with all other strains. The effects of crossing over in F_1 females as well as the effects of assortment of whole chromosomes by using F_1 males were observed by testing 450 recombinant and non-recombinant second and third chromosomes for a total of 1800 chromosomes.—The average viability was increased by recombination in homozygous second chromosomes, but decreased

in third and double homozygotes. Segregation of whole chromosomes acted to decrease viability slightly for chromosome II but greatly for chromosome III presumably by producing newly assorted nonselected combinations of chromosomes. Genetic variance was greater from the effects of crossing over than for assortment of whole chromosomes. Statistical analyses show interaction of linked loci and interaction between whole chromosomes to be highly significant in determining viability. There seems to be a qualitative difference between second and third chromosomes in their response to interchromosomal recombination: new combinations of whole third chromosomes consistently lower viability while whole second chromosomes do not do so significantly. The fact that intrachromosomal recombination tends to increase viability on II but decrease it on III may reflect an approach to the average viability of chromosomes in the original population where chromosome II has higher viability than III. The number of lethals produced in this experiment is less than that to be expected from mutation and does not show evidence of recombinational origin ("synthetic lethals"). Genetic variability released by crossing over while highly significant is not diverse enough to produce extremes of viability however, so that lethals would not be expected. Intrachromosomal recombination between normal viability chromosomes accounts for 32% for II and 34% for III of the total variability of nonlethal chromosomes observed in the original population. Interchromosomal recombination accounts for the following proportions: zero for II and 11% for III. (Work done under Contract AT[30–1]-1775, U.S. Atomic Energy Commission.)

ALLEN, MARCIA, and CHARLES YANOFSKY, Stanford University, Stanford, Calif.: *A study of reversion in the A gene (tryptophan synthetase) of Escherichia coli.*—Reversions in the *A* gene (tryptophan synthetase) are being studied since the *A* gene-A protein system can be examined at the fine structure level of both gene and protein. The *A* mutants employed, all ultraviolet induced, were found to differ widely in their reversion rates and patterns. Some gave only "wild-type-like" revertants while others yielded, in addition, several classes of partial revertants. The different types of revertants were distinguished on the basis of one or more of a number of tests, including colony size determination, enzyme activity of cells, 5-methyl tryptophan sensitivity, enzyme activity of cell-free extracts and genetic tests.—Some of the *A* mutants employed form an altered A protein, while others show no trace of A protein. Both types yield full revertants and partial revertants. The A proteins from the partial revertants examined are still somewhat defective in catalytic ability, showing that partial reversion does not restore the normal protein.—Reversion experiments with 2-amino purine and 5-bromouracil showed, in agreement with the findings of others, that these base analogs increase the reversion frequency of only certain mutants. In addition 2-amino purine does not increase the reversion frequency of all classes of revertants from each mutant but only certain ones, depending on the strain examined. Furthermore, of three distinguishable mutants(different abnormal A proteins) altered at the same site in the *A* gene, only one responded to 2-amino purine. These findings will be discussed in terms of our present concept of mutation.

ALLEN, SALLY LYMAN, University of Michigan, Ann Arbor, Mich.: *A first case of linkage in the ciliated protozoa.*—The haploid chromosome number of *Tetrahymena pyriformis* is five; thus, on *a priori* grounds detection of linked loci should be relatively easy. Six loci have been described in variety 1. The *mt* locus, with six alleles, is concerned with the expression and frequency of the seven mating types. The *H* locus, with five alleles, controls the ciliary antigens produced at 25°C. The *mt* and *H* loci are unlinked. Recessive alleles at the *F* and *T* loci loci govern traits "fat" and "tiny," which are lethal following conjugation. *F* and *T* are unlinked. The *E-1* and *E-2* loci, each with two alleles, specify different types of esterases, revealed by starch gel electrophoresis. *E-1* and *E-2* are unlinked. An analysis of the linkage relationship of the *E-1* and *mt* loci shows that they are linked, with about 25% crossing over. Testcrosses of the *mt* genotype of 40 backcross clones involving strains B and C have been completed, but testcrosses of clones with the "homozygous" E-1 phenotypes (E-1B and E-1C) have not been made. Some of these could be heterozygotes, since the heterozygote can differentiate to express either

E-1B or E-1C. Four types of backcrosses yield a total ratio of 18*a*:4*b*:6*c*:12*d*. Classes *a* and *b* can be definitely classified as *E-1* heterozygotes, since they were E-1B + C in phenotype. Classes *c* and *d* were E-1B or E-1C in phenotype. Although an accurate measure of crossing over is not possible at present, the establishment of linkage constitutes the first known case in the ciliated protozoa. (Supported by grant C-3545 from the National Cancer Institute, U.S.P.H.S.)

ANNAN, MURVEL E., and PHILIP J. REITAN, Wagner College, Staten Island, New York: *Effects of desiccation of Drosophila females on the frequencies of irradiation induced embryonic abnormalities.*—Virgin *D. melanogaster* females were desiccated immediately prior to exposure to 4,000r units of gamma irradiation from a cobalt-60 source. The effects of this treatment were observed on egg hatchability. The results confirm the findings of HERSKOWITZ in that desiccation enhances the irradiation induced reduction in egg hatchability. In addition, observations were made on late stage embryos (16 or more hours of development) in order to determine whether or not the desiccation effects could have been due to effects on fertilization. The embryos were classified as either (1) normal, (2) grossly abnormal, or (3) showing no development. Preliminary results indicate that there is no difference in the developmental picture between embryos from irradiated flies whether they were desiccated or not. Further observations are being made to substantiate these results and to determine whether or not more subtle embryonic abnormalities might be contributing to the desiccation effects on irradiation induced "dominant lethals." (This work was supported by grant A-2162[C3] from the National Institute of Health.)

BACON, D. F., Institute of Microbiology, Rutgers, The State University, New Brunswick, N. J.: *Rare Escherichia coli diploids revealed by cross-feeding following conjugation.*—Certain slow growing (*S48*) and fast growing (*F24*) revertants of an acetylornithinaseless mutant (*orn*$^-$) of strain W represent mutations at or near the *orn*$^-$ locus; *S48*, but not *F24*, revertants crossfeed another ornithine-requiring mutant, 39A-23. The markers, *orn*$^-$, *S48*, and *F24*, lie close to a methionine (*meth*$^-$) locus. In experiments designed to detect recombination between the *S48* and *F24* sites, a double-step revertant, *orn*$^-$ →*S48* →*F24*, was crossed with *orn*$^-$ Hfr; the recipient also carried *meth*$^-$ and a proline marker (*pro*$^-$), and the donor, a tryptophan marker. Ornithine- and methionine-independent exconjugants were selected on minimal agar supplemented with proline and layered above minimal agar seeded with cells of 39A-23. Most recombinants are fast growing and do not crossfeed; however, two out of 20,000 recombinant colonies gave slow growth and crossfeeding, and proved to contain diploids, heterozygous in the *orn*$^-$ *meth*$^-$ region, and hemizygous or homozygous for *pro*$^-$. These heterozygotes were maintained on minimal agar supplemented with proline; for each transfer, one of the numerous small colonies obtained was subinoculated. An analysis of the cell types present in these small colonies yielded 56% heterozygotes and the following segregant types: 32% *orn*$^-$*meth*$^+$ (donor parent), 10% *orn*$^+$*meth*$^-$ (recipient), 1.5% *orn*$^+$*meth*$^+$, 0.5% *orn*$^-$*meth*$^-$, *orn*$^+$ denoting ornithine-independence of the *F24* type. These percentages remained constant over 20 subinoculations. (Aided by the U. S. Public Health Service and the Office of Naval Research.)

BAND, H. T., University of British Columbia, Vancouver, Canada: *Viability of second chromosome heterozygotes of Drosophila melanogaster in constant and fluctuating environments.*—Drastic (le + sle) chromosome frequency in the S. Amherst population of *D. melanogaster* shows significant negative correlation with temperature range and somewhat positive correlation with mean temperature (BAND and IVES, 1961). Second chromosomes from a September 1960 sample of this population providing the genetic material, the viability of 30 drastic/drastic, 40 drastic/nondrastic, and 30 nondrastic/nondrastic heterozygous chromosome combinations are being compared at C77 (= constant 25°C), C62.6 (= constant 17°C) and four fluctuating environments — a wide and a narrow range between daily maximum and minimum temperatures with means both at 77° and 62.6°. Matings between any two chromosome lines are $Cy/i \times Bl/j$ such that the *i*/*j* wild-type progeny carry one of the three combinations; three replications of each mating are scored in each environment. Males of 20 drastic/nondrastic (le/qn and le/n)

combinations are then utilized for rederivation experiments to determine if gametic contribution to the next generation is also subject to environmental modification. The fluctuating environments are produced by a 24-hour, cam-controlled incubator.—Experiments at C77 and F64/90 (wide range, mean 77°) have just been completed. Although viability differences at C77 are not significant, drastic/drastic combinations give highest viability (% +/+ = 29.7). At F64/90 viability differences are significant, nondrastic/nondrastic combinations being superior (% +/+ = 33.6). In both environments significantly more lethal-bearing gametes are recovered from le/non-le males than nonlethal bearing, 70% from C77 males, 61% from F64/90. These results indicate that while warmer environments favor lethals, wide temperature fluctuations depress the viability of their carriers. Additional aspects of the data and results at the other four environments will be presented. Their bearing on the observations from natural populations and on the role of lethals and semilethals in the genetic structure of populations will be discussed. (Supported by grants from the National Science Foundation and the National Research Council of Canada.)

BARON, L. S., and STANLEY FALKOW, Walter Reed Army Institute of Research, Washington, D.C.: *Genetic transfer of episomes from Salmonella typhosa to Vibrio cholerae.*—Episomes, a class of genetic factors existing in the bacterial cell independently of the chromosome, are usually transferable to other cells following cellular contact. Initially described by JACOB and WOLLMAN in *Escherichia coli*, our results have extended the occurrence of episomes to other enteric bacterial genera. An episomic element controlling lactose utilization, termed F°-*Lac*, found in a strain of *Salmonella typhosa* and its transfer to other Salmonella, Shigella, and Escherichia strains have been reported (FALKOW and BARON, Bacteriol. Proc. 1961). Transfer of F°-*Lac* from *S. typhosa* to *Serratia marcescens*, an organism of significantly different DNA base ratio than *S. typhosa*, a basis for chromosomal genetic incompatibility, has been accomplished also (CAREY, SPILMAN and BARON, Bacteriol. Proc. 1960; FALKOW *et al.*, in manuscript). We now report the transfer of lactose utilization from *S. typhosa* to *Vibrio cholerae*, an organism not even classified in the enteric group, as well as of different DNA base ratio. Transfer of lactose utilization to *V. cholerae* was obtained using either the F-*Lac* episome of JACOB and WOLLMAN or the F°-*Lac* episomic strain of *S. typhosa*. It has been possible also to transfer the episomic factor for antibiotic resistance (MITSUHASHI *et al.*, Japan. J. Exp. Med. 39: 179, 1960) from *S. typhosa* to *V. cholerae* and to strains of *V. cholerae* already containing the F-*Lac* episome. *V. cholerae* possessing F-*Lac* or F°-*Lac* can act also as donors for these episomes in crosses with *Salmonella typhimurium* as recipient strain.

BARRATT, RAYMOND W., Dartmouth College, Hanover, N. H.: *Studies on gene-protein relations with glutamic dehydrogenase in Neurospora crassa.*—Mutants at the amination locus (*am*) lack glutamic acid dehydrogenase (GAD) activity, as shown by FINCHAM. GAD has been isolated, purified, and characterized from wild type 74A. Under suitable growth conditions 1% of the protein extractable from mycelia is GAD (aerated submerged culture, 35–37°C, heavy inocula giving 2 to 5 $\times$ 10^6 conidia per ml, initial nitrogen starvation, 24-hour growth period).—GAD has been purified by homogenization (frozen mycelium, 0.1M phosphate buffer *p*H 7.0, Waring and Virtis homogenizers), heat treatment (52°C for 30 minutes) fractionation with ammonium sulfate, salt gradient elution on DEAE cellulose columns. Purified GAD migrates electrophoretically as a single band on cellulose acetate at 3 *p*H's, in starch gel and shows only two minor components in cyanogum. Preliminary ultracentrifuge data indicate a molecular weight of about 200,000–250,000 (S = 11.5). Twenty-six different peptides are obtained when a tryptic digest is fingerprinted. The Sakaguchi test shows nine or ten arginine positive spots, each of which corresponds to a peptide. Total amino acid analysis of an acid hydrolyzate gives a ratio of 15 lysines to nine arginines, which could account for 25 of the peptides on trypsin digestion and nine arginine containing peptides. On the basis of the amino acid ratios found, a subunit containing 15 lysine residues would contain a total of 243 residues, with a molecular weight of less than 30,000.—GAD isolated and purified from partial reversions at the *am* locus

will be compared with wild type GAD. Several of the *am* mutants have been shown to produce a protein which purifies in the same fraction as GAD. (Supported by a grant from National Science Foundation and a Special Research Fellowship from National Institutes of Health.)

BARTLETT, ALAN C., and A. E. BELL, Population Genetics Institute, Purdue University, Lafayette, Indiana: *Radiation effects on reproduction in plateaued and unselected strains of Tribolium castaneum.*—The effects of various levels of X-radiation on reproductive fitness of a plateaued population of *Tribolium castaneum* and its unselected base population were investigated. The plateaued population had been selected for increased body weight for 44 generations and weighed approximately 5500 μg in contrast to 2100 μg for the unselected strain. Two components of fitness (eggs laid during a standard 48-hour period and number of resulting progeny) were observed. While the two strains did not differ in egg production, the unselected strain had superior hatchability. Within each of nine doses (ranging from 200r to 5000r) the beetles were partitioned so that in one set only males were irradiated, in another set only females and in the third both males and females were irradiated.—Irradiation of males was shown to have no effect on the ability to excite egg laying in their mates, but number of progeny was reduced with the higher doses. Egg production was not affected for doses up to 1000r applied to females but a marked decrease was seen beyond that level. A decline in number of progeny from irradiated females was observed at doses as low as 600r. The initial superiority of the unselected strain for number of progeny persisted over-all doses.—A linear response to irradiation was not shown for either egg production (somatic effects) or number of pupae hatching (somatic effects and dominant lethal effects) for the dose range employed in this study. (Work supported by grants from the Atomic Energy Commission and the National Science Foundation.)

BAUMILLER, ROBERT C., S. J., Saint Louis University, St. Louis, Mo.: *Prepupal effects of X-ray-induced, euploid and near-euploid mutants in heterozygous condition in Drosophila melanogaster.*—The effect of euploid and near-euploid heterozygous mutants in *D. melanogaster* has already been studied in the egg and larval stages separately. The present work studies both stages in the same individual in order to estimate the minimal total effect of such mutants.—Wild-type, Oregon-R males were treated (I) or not (C) with 3000r or X rays and mated to virgin, untreated, sibling females. Eggs were collected at half-hour intervals, and each hour, starting the 21st hour after oviposition, the numbers of larvae hatching were scored. Of all larvae, the percentages hatching late (more than one hour after the largest hourly percentage hatched in C) were: C, 2.9 ± 0.3 (of 2950 larvae); I, 8.8 ± 0.9 (of 1057 larvae). The *induced* difference in delay, of $6.1 \pm 0.9\%$, is attributable to newly arisen (near-) euploid mutants in heterozygous condition. Not-delayed larvae (obtained from the hour when the largest percentage hatched in C) and delayed larvae were studied for retarded pupation and for death before pupation. The *induced* difference between not-delayed C and I was $9.7 \pm 0.8\%$ for retarded pupation and $6.7 \pm 0.7\%$ for death before pupation. Among delayed larvae the *induced* difference between C and I was $23.6 \pm 0.7\%$ and $19.4 \pm 0.7\%$, respectively. Thus, about 16% $(6.1 + 9.7)$ of the larval population which carry X-ray-induced, euploid and near-euploid, heterozygous mutants show a detrimental effect of these mutants in either the egg or the larval stage, of which approximately 1.4% were affected in both stages. (This investigation was carried out during the tenure of a Predoctoral Cooperative National Science Foundation Fellowship, and supported by a grant to I. H. HERSKOWITZ and associates from the U. S. Atomic Energy Commission (Contract AT[11–1]-633). Present address: Department of Medical Genetics, University of Wisconsin, Madison, Wis.)

BENDER, H. A., University of Notre Dame, Notre Dame, Ind.: *Distribution of periodic-acid-Schiff-positive substances in the oocyte of Drosophila melanogaster.*—Comparisons were made of wild-type and various singed and lozenge mutants. Flies were sacrificed at seven days, abdomens removed and fixed in Kahle's fluid, dehydrated in dioxane and infiltrated and imbedded in paraffin. Complete serial sections of more than 20 individuals of each category were studied.

Glycogen could be demonstrated, following the methodology of BONHAG (J. Morph. 99: 433–464, 1956), throughout the fully grown, stage 14, oocyte of wild type flies but was not detectable in the female-sterile sn^{36a} mutant.—Preliminary considerations as to the origin and distribution of glycogen and other PAS reactive substances of the oocyte will be presented. (Contribution from the Radiation Laboratory operated by the University of Notre Dame and supported in part under U. S. Atomic Energy Commission contract.)

BENDER, M. A, and P. C. GOOCH, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Chromosome aberrations in human blood irradiated in vitro.*—Because it appears to approximate the case of human cells irradiated *in vivo* more closely than any other system, we have studied the chromosome aberrations induced in peripheral leukocytes when human blood is irradiated *in vitro* just after it is drawn. The leukocytes were prepared for cytological examination by a modification of the method of MOORHEAD, *et al.* (Exptl. Cell Research 20:613, 1960). The control aberration rate was about 0.02 breaks per cell. No aberrations that could be positively identified as chromosome types were found. While we were unable to show that any chromatid-type aberrations were induced by X-rays, we found that chromosome-type aberrations were induced at a rate of about 0.004 breaks per cell per r at low doses. Rings, dicentrics, symmetrical translocations and deletions of both the terminal and the interstitial types were found. This observed rate of X-ray-induced chromosome breakage is about the same as that observed previously in other systems. The fact that only chromosome-type aberrations were seen in the X-rayed material shows that most if not all of the circulating leukocytes that are able to divide in our *in vitro* system are in the pre-DNA-synthesis (G_1) growth phase at the time the blood is drawn.

BEN-ZEEV, NECHAMA, and ZAMENHOF, STEPHEN, Department of Biochemistry, Columbia University, New York, N.Y.: *Effects of high temperatures on dry seeds.*—Heating of dry bacterial cells and spores to high temperatures *in vacuum* induced a high frequency of mutations (ZAMENHOF, Proc. Natl. Acad. Sci. U.S. 46:101, 1960). The treatment was designed to inflict genetic damage to DNA without excessive lethality to the cell (protection of protein by drying *in vacuum*). The purpose of the present work is to extend these studies to higher plants. Dormant seeds of *Raphanus sativus*, *Brassica napus*, *Nigella damascena* and *Hordeum vulgare* were dried and heated (16 min) *in vacuum*. Survival was obtained at temperatures 122° up to 138°C which was up to 35°C higher than without vacuum. Such treatment produced delay and inhibition of germination for more than four months (Nigella), both removable by gibberellic acid and, to a lesser degree, by kinetin but not by thiouracil. Pronounced morphological changes in the plant were also produced. Cytological study of the roots tips (Nigella, 118–122°C) revealed the presence of micronuclei, a lower number of chromosomes, their stickiness or point adhesions of chromosomes at anaphase, breakage in the centromere region, as well as the appearance of small and large chromosome fragments, translocations and bridges. Some of these chromosomal changes may represent genetic injuries, either viable or lethal.

BIANCHI, A., Plant Genetics Institute, Catholic University, Piacenza, Italy: *Duplication of specific chromosome segments to increase kernel weight in maize.*—Nondisjunction of B^A chromosomes in the second microspore division of maize stocks carrying interchanges between A and B chromosomes results in kernels possessing embryo and endosperm with different chromosome complements. Appropriate crosses permit one to recognize promptly hypoploid, hyperploid as well as the normal types of embryo and endosperm, for several of the available TB-A stocks. It has been possible to demonstrate that TB-4 gives rise to hyperploid endosperm whose weight is about 20% higher than the normal one. Although several points need further study and confirmation, the finding appears of significance in maize breeding.

BIANCHI, A., and M. CONTIN, Institute of Genetics, University of Milan, Italy: *Changes in radiomutability of maize seeds with various moisture content.*—Maize seeds heterozygous for

alleles of yg^2 locus were X-rayed with 6 kr at room temperature after having been kept in desiccators or in water, for different time periods, at about 13°C, in order to obtain embryos with moisture contents ranging from about 4 to 30%. Somatic sectors, in leaves 3rd–5th, representing losses of the dominant allele(s), were counted to provide an index of induced mutability. Sector frequency was very high in seeds possessing less than 6% of moisture content. A plateau was exhibited by seeds with values between 7 and 20% while the radiosensitivity increased again with still higher water content.

BLAIR, PAUL V., W. H. KYLE, V. L. ANDERSON, and A. E. BELL, Population Genetics Institute, Purdue University, and Agricultural Research Service, USDA, Lafayette, Indiana: *Quantitative consequences of mating systems and selection methods.*—A foundation population of *Tribolium castaneum* was formed by crossing three wild stocks and one mutant stock. The trait investigated (14-day larval weight) exhibited 33.7% heterosis in the two-way crosses and 39.4% heterosis over the parent stocks in the four-way crosses. Three generations of random mating preceded the experiment, and heritability of larval weight was estimated to be .202 in this foundation population.—All combinations of two selection methods (individual and random) and five mating systems (inbreeding, outbreeding, phenotypic assortative, phenotypic disassortative and random) were investigated in two replications through eight generations. All subclasses in each replication were initiated with identical pedigrees. In each generation, 120 individuals per subclass were weighed from a maximum of 20 single-pair matings.—The average response of larval weight to individual selection was 60.7 micrograms per generation, and the realized heritability estimates ranged from .050 to .282. In the eighth generation, the descending ranks of the mean larval weights in the mating systems under individual selection were: outbreeding, phenotypic assortative, random, phenotypic disassortative and inbreeding.—Highly significant effects of mating systems and selection methods were obtained in each generation. The only significant change in variance was observed in the inbred mating system.—Crosses between the two replicates of the selected outbreds of generation eight were significantly larger than similar crosses between the selected inbreds. (This work was supported in part by a grant from the National Science Foundation.)

BLUMEL, JOHANNA, Department of Surgery, Division of Orthopedics, University of Texas Medical Branch, Galveston, Texas: *Hereditary factors in congenital skeletal anomalies of the spine.*—Anomalies of the spine are of etiological and clinical significance because of their importance in regard to treatment and pathogenesis.—This is a preliminary report on a study undertaken to elucidate the hereditary factors of congenital anomalies of the spine.—Vertebral anomalies in 264 patients, 141 male (120 Caucasian and 21 Negro) and 123 female (101 Caucasian and 22 Negro) were grouped into seven categories according to what was considered the main or primary defect.—The most frequently encountered spinal anomaly was spina bifida with 88 cases, 53 male and 35 female. Numerous associated anomalies were present in this group.—There were 61 patients with spondylolisthesis, 40 male and 21 female.—Idiopathic scoliosis was present in 45 patients, 13 male and 32 female.—Thirty-three patients had transitional vertebrae, 18 male and 15 female.—Hemivertebrae was present in 17 patients, six male and 11 female.—Complete or partial agenesis of the sacrum and coccyx was present in 14 patients, six male and eight female.—Failure of segmentation was present in six patients, five male and one female.—The sex ratios in scoliosis, hemivertebrae, and sacral anomalies showed an excess of females.—Family studies on some of these patients will be discussed. (This research was supported by Grant CRMS 176 from The National Foundation.)

BRAVER, G., University of Oklahoma, Norman, Oklahoma: *Studies of a possible intrachromosomal effect on crossing over in the X chromosome of Drosophila melanogaster.*—During matings designed to study the effects on crossing over of heterochromatin added to the distal end of the X chromosome, an apparent intrachromosomal effect was observed. With X chromosomes heterozygous for the medium sized AB inversion, increasing reductions in distal (w^a–*cv*–bk. pt.) ex-

change following addition of FR-1 (Y^S) heterochromatin to first one and then both of the X chromosomes was accompanied by increases in proximal (*f-car*) exchange. Proximal exchange was highest in those crosses with lowest distal exchange, where both X chromosomes carried the extra distal heterochromatin.—The results of present studies on the effects of varying both the distal heterochromatin and the inversion will be reported. (Work supported in part by the National Science Foundation.)

Bray, David F.,[1] The University of British Columbia, Vancouver 8, Canada: *The effect of size and restrictions on inbreeding and equal representation on minimizing variation of control populations.*—Three complete replications of a 2^3 experiment were carried out over seven generations with *Tribolium castaneum.* The two levels of each of the three factors investigated were: inbreeding, no restriction or no full sib-matings permitted; equal representation, no restriction or restricted such that each family contributed equally to the next generation; size of breeding population, eight single pair matings or 16 single pair matings. Mass matings were also carried for each size level.—The grand average pupa weight for all ten treatments was computed for each generation. The difference between each treatment average and the grand average was then computed within generation. Although the distribution of this variable is unknown, the central limit theorem permits the analysis of variance of their means over generations. The treatment combinations having the smallest mean deviation from the grand average are considered to be more useful as controls.—Although considerable variation between replications was observed, both size and equal representation were shown to account for nearly all of the treatment variation. Either doubling population size from eight to 16 pairs or imposing the equal representation restriction with eight pairs resulted in a reduction of over 25% in mean deviation. The use of the equal representation restriction with 16 pairs reduced the mean deviation by 47% compared to no restrictions with eight pairs. ([1] Now with the Genetics Section, Animal Research Institute, Canada Department of Agriculture, Central Experimental Farm, Ottawa.)

Brewbaker, James L., and George C. Emery, Brookhaven National Laboratory, Upton, New York: *Radiation-induced aberrations and the structure of the incompatibility genes.*—A high degree of multiple allelism (several hundred alleles per species) typifies the incompatibility (*S*) locus which occurs in perhaps 10,000 angiosperm species; each *S* allele produces unique stylar and pollen products analogous to antigens and antibodies; only rare modifiers are known to influence the specificity of product interaction. The thesis that the *S* locus is a composite of subunits, potentially separable by crossing over or mutation, could comfortably embrace these and other facts, and has been subjected to examination.—Mutants sought from among roughly 30 million petunia pollen grains included no new *S* alleles. No "pollen-part" or "stylar-part" changes involving exclusively one or the other aspect of the gene action were recovered. Crossing over between closely linked units reasonably may be expected to create identifiable duplication- or deficiency-genotypes in this system; it was not observed among the five alleles tested (resolution 0.02 unit). The 270 "mutants" cytogenetically tested were derived largely from premeiotic X-irradiations, and included four self-fertile types: *S*-locus bearing centric fragments (90% of mutants), tetraploids, deletions and modifiers (plus one probable translocation). The absence of "point mutants" to functional new alleles is rationalized as a consequence of the evident multiple-stranded complexity of higher plant chromosomes.

Brody, Stuart, and Charles Yanofsky, Stanford University, Stanford, Calif.: *The restoration of a wild-type-like protein by suppressor gene action.*—Suppressed strains derived from three different tryptophan synthetase "A mutants" of *E. coli* were examined in a study of the mechanism of suppressor gene action. Each of the unsuppressed mutants employed forms an altered A protein which is active only in the indole → tryptophan reaction, while the wild-type A protein is active in the physiologically essential reaction, indoleglycerolphosphate → tryptophan, as well. The altered A proteins of these mutants also have different physical properties

than the wild-type A protein. Examination of the A protein fraction from the three suppressed mutants revealed the presence of low levels of indoleglycerolphosphate → tryptophan activity. In all three cases this activity was found to be associated with a protein with the same physical properties as the wild-type A protein. Furthermore, the bulk of the A protein in these suppressed mutants had different physical properties than the wild-type A protein and was either heat-labile, acid-labile, or heat-stable, and indistinguishable from the A protein of the respective mutants. The presence of two A proteins in the suppressed mutants was also shown by chromatography on DEAE cellulose columns. The purified "suppressed A protein" from one strain also had the same physical properties as the wild-type A protein. These findings strongly suggest that the primary structure of a gene product, the A protein, can be changed by specific suppressor genes. Possible mechanisms will be discussed.

Brosseau, George E., Jr., State University of Iowa, Iowa City, Iowa: *Non random recovery of detachments from an attached-X chromosome in Drosophila.*—Irradiation of females of the constitution y^s *su-w*^{a}*w*^{a}*bb*:=/B^SY $y+$ with 990r of X rays yielded 159 detachments. Of these 57 carried B^S, 58 were $y+$, 32 carried neither marker and 12 were sterile or were lost before testing was completed. The detachments that carried neither marker were tested further for evidence of involvement of the Y in the exchange and none was found. These are probably autosomal detachments and were not tested further. The remainder of the detachments were tested to determine the location of the Y break with respect to *bb*+ and the fertility factors. Complementary detachments were recovered in very unequal numbers. The exchange resulting in a detachment produces an asymmetric dyad from which the shortest element is recovered more frequently. This is similar to those cases in which Novitski (1951) found non random disjunction, and it is likely that this is another case of the same phenomenon. The coefficients of non randomness for three classes of detachments are: 0.76, 0.88, 0.71. Fifty-three of the 115 breaks or 46.1% were between the most distal fertility factor of the arm involved and the X-derived terminal marker. Another peak of breakage occurs in the portion of Y^S that is homologous to the X. Perhaps most of the exchanges that produce detachments occur in synapsed homologous regions and detachments with the Y might be induced crossovers rather than translocations. (Supported by research grant No. RG-6508 from the National Institutes of Health, U. S. Public Health Service.)

Brown, E. H., and R. C. King, Northwestern University, Evanston, Ill.: *The occurrence of ovarian tumors in Drosophila melanogaster heterozygous for an unstable chromosome.*—During normal oogenesis about one developing egg chamber in 20,000 becomes tumorous. Irradiation of newly emerged females with 4,000r of Cobalt-60 gamma rays increases the frequency of tumors about 26 times. Ionizing radiation is known to result in chromosome breakage and subsequent loss of the acentric fragments. This fact suggests that deletion of segments and chromosomal elimination may lead to the production of tumors. To check this point a study was made of the reproductive systems of individuals heterozygous for an unstable, ring X chromosome. A total of 3893 egg chambers from 53 ovaries (representing 31 gynandromorphs) were examined, and 125 (3.21%) of the chambers were found to be tumorous. These tumors were distributed among 11 of the gynandromorphs examined. The gynandromorphs were obtained by crossing *In(1)*X^{c2}, w^{vc} / y w^{m4} females to y w^{m4} males. Cytological study of the tumors indicates that at least some arise through the invasion of the egg chamber by cells arising from the follicular envelope, as tongues of these cells are often observed which extend from the follicular epithelium into the chamber. In cases where the chamber is filled with small tumor cells (and no nurse cells or oocyte are present) the tumor may be of germ cell origin. A study of the karyotype of the tumor cells is under way. (Research supported by Grant No. GF 13,487 from the U. S. Public Health Service.)

Brown, Wm. P.,[1] and A. E. Bell, Purdue University, Lafayette, Indiana: *Control populations in selection experiments for egg number in Drosophila melanogaster.*—The adequacy of a

control in selection experiments rests upon its ability to change in the direction and to the extent only that the experimental population fluctuates due to the environment. An experiment designed to compare alternative methods of selection for increased egg number in plateaued populations (BROWN and BELL, Genetics 45:979, 1960) provided an opportunity to evaluate randombred populations on their ability to reflect environmental effects.—A randombred was derived from each of two plateaued populations which were homozygous for a different recessive mutant. Two samples of eggs were taken from each randombred when they served as controls. One was placed into appropriate experimental cultures of the reciprocal recurrent method of selection since its features allowed the progeny to be distinguished phenotypically upon emergence. This mingling during development facilitated the measurement of pre-emergence environmental effects which could influence egg number in the adult female. The other sample developed separately in a conventional manner. Thus, four different controls were maintained: two randombreds each reared by two methods.—The various controls revealed, equally well, environmental trends over the ten generations of the experiment. However, the two randombred populations differed significantly both in the direction and in the extent of intergeneration fluctuations. There was no significant difference between the methods of rearing. The value of a control seems to depend more upon its genetic constitution than the method of rearing. (Supported by a grant from the National Science Foundation.) (1 Now at The University of Connecticut, Storrs, Connecticut.)

BROWNING, LUOLIN S., and EDGAR ALTENBURG, Rice University, Houston, Texas: ***The correlation of the ratio of lethal to visible mutations with that of whole-body to fractionals induced by X-rays and chemical mutagens.***—In experiments designed to test the relative rates of lethal and visible mutations induced by X-rays and chemical mutagens (using MULLER's Maxy technique) as well as the relative proportion of whole-body and fractional mutations among the visibles, it was found that in the X-ray series, the ratio of lethals to visibles was much higher than in the case of the chemical mutagens: 78.1 lethals:1 visible after treatment of the adult males with 3,000r *vs.* 8.7 lethals:1 visible for dimethyl myleran, 12.9:1 for phenylalanine mustard, and 19.9:1 for ethyleneiminohydroquinone. However, these proportions of lethals to visibles were directly correlated with the ratio of whole-body mutations (visibles) to fractionals, being highest for X-rays (113:9 in 159,000 tested chromosomes), lowest for dimethyl myleran (13:26 in 17,000 chromosomes), next lowest for phenylalanine mustard (11:12 in 24,000 chromosomes), and next for ethyleneiminohydroquinone (21:9 in 31,000 chromosomes). It is concluded (as suggested by CARLSON and OSTER) that gonadal lethal mosaics account in large measure for the relatively low rate of detected lethals to visibles in the chemical series, a conclusion in agreement with the earlier work of AUERBACH showing that gonadal mosaicism is higher under chemical treatment than under X-rays. (Larger scale tests by us are in progress, similar to those of AUERBACH). The present experiments further indicate that the ratio of fractionals to whole-body mutations induced by X-rays is somewhat dose-dependent, being higher for 5,000r (18:95 in 4,900 tested chromosomes) than for 3,000r (9:113 in 7,700 tested chromosomes). In terms of MULLER and CARLSON's "rotational substitution" theory, this result might indicate that the higher dose (5,000r) might have caused a relatively higher proportion of breakage of base pairs into single bases with a resultant lower proportion of "rotational substitutions" of the intact base pairs and relatively more single base substitutions. (This work has been supported by grant C-3909 from the National Institutes of Health, U.S. Public Health Service.)

BROWNING, LUOLIN S., and EDGAR ALTENBURG, Rice University, Houston, Texas: ***The linearity of the ultraviolet dose-rate curve after photoreactivation in Drosophila.***—It has been previously shown that when the polar cap cells of Drosophila are treated with ultraviolet and the lethal rate induced in the second pair of chromosomes detected (by means of MULLER's sifter technique), the dose-rate curve rises rapidly at the lower doses employed. With further increase in dose, it becomes less steep and reaches a plateau when the dose is large enough to give a

detectible frequency of 5% to 10%. It then drops. In the present experiments the polar cap cells were posttreated with photoreactivating light after treatment with ultraviolet over the same dosage range as in a non-posttreated series. In the posttreated series it appears that the dose-rate relationship best fitted a linear curve up to a point beyond that at which the "unphotoreactivated" rates started to drop (with increasing dose), the photoreactivated rates being well below those of the non-posttreated at the lower dosage ranges ($1.3 \pm 0.8\%$ *vs.* $3.5 \pm 1.0\%$), when the non-posttreated rate is on the steeply rising part of the dose-rate curve, the two finally becoming about equal at our highest doses ($3.8 \pm 0.7\%$ *vs.* $4.5 \pm 1.5\%$), when the non-posttreated rate is on the falling part of the curve. (This work has been supported by grant C-2393 from the National Institutes of Health, U. S. Public Health Service.)

BUTLER, L., University of Toronto, Toronto, Canada: *Changes in the monogenic ratios of the tomato gene rosette (ro).*—Rosette was produced by X-radiation in 1930. It is a recessive and the homozygote has never flowered, therefore no simple selection for increased viability can be practised. The early monogenic ratios were 437:87 which gives 16.6% *ro* instead of the expected 25%. Outcrosses to dwarf (d_1), showed definite linkage with a crossover value of 18.5% and a monogenic ratio of 5076:1004 which demonstrates a similar deficiency of rosette. When outcrossed to another second chromosome gene, green stem (*aw*), the seven crosses showed no heterogeneity and had a monogenic ratio of 14,623:2623. These crosses showed a similar shortage of rosette plants and a crossover value of 15% between *ro* and *aw*. F_2 selections of the genotype *aw aw Ro ro* were used to produce five coupling phase crosses, and these showed heterogeneity of the monogenic ratios. One cross had a ratio of 1206:244 or 16.8% *ro*, whereas the other crosses had a ratio of 2456:775 or 24% *ro*. Thus it appears that there are two different *aw aw Ro ro* selections, one with high viability and one with low. It is suggested that *ro* is the result of a chromosome deficiency and that a crossover in this region has led to partial restitution. The genes, *suf* and *dil* lie in this region of the chromosome, and crossovers involving these genes are being examined for similar effects on the monogenic ratio.

CALDECOTT, RICHARD S., and D. T. NORTH, University of Minnesota, St. Paul, and Agricultural Research Service, USDA: *Irradiation induced reversion to near normal phenotype of an X-ray induced chlorophyll mutation.*—An X-ray induced recessive yellow virescent mutation in Himalaya barley differs in intensity of pigmentation from the normal at all stages of growth. Furthermore, when mutant seedlings are grown at 13°C or below they do not develop green pigment which characterizes the normal.—In two separate years, a total of 17,226 mutant seeds were subjected to either X-rays or thermal neutrons and then grown to maturity under isolation. Up to five heads were removed from each surviving plant and head progenies grown in a greenhouse maintained at approximately 13°C. At emergence, and thereafter until the first leaf was fully extended, screening was done to ascertain whether or not green pigment developed on any seedlings.—One or more green seedlings appeared in 176 head progenies. The intensity of pigmentation ranged from nearly as green as the control to those with only a trace of green pigment. At maturity the fertility of the revertants ranged from 0 to 100% with the bulk of the material evidencing a seed set between 50 and 75% of the control. No revertants were found in 7,681 head progenies of the control.—Based on X_1 and X_2 analyses of seed set and/or segregation ratios four conclusions can be drawn. (1) The revertants are probably dominant changes. (2) Degrees of reversion towards normal take place because quantitative differences in chlorophyll occur in different revertants. (3) Some reversions are probably associated with chromosome structural changes, this being the most likely explanation of the sterility in some of the material. (4) The frequency of reversion is much greater than anticipated, based on published back mutation data in Neurospora.

CARLSON, E. A., and I. I. OSTER, University of California, Los Angeles, California, and Institute for Cancer Research, Philadelphia, Pennsylvania: *Chemical-mutagen induced mosaicism at the dumpy locus.*—Injection of a monofunctional quinacrine mustard, 2-methoxy-6-chloro-

9-(3-(ethyl-2-chlorethyl)amino-propylamino)acridine dihydrochloride, previously shown to be mutagenic (OSTER and POOLEY, Genetics 45:1004–1005, 1960) into the abdomens of *sc*^{8}-Y-*B*^{8}/*y*^{2} *w*^{1} *ct*^{6} *f*; +; + males was followed by brooded inseminations of *y sc*^{81} *In49 sc*^{8}; *dp bw*; *st p*^{p} females. Examination of the F_1 progeny derived from sperm released 1, 3, 5, and 7 days after treatment yielded induced mutation rates of 0.0 (0/895); 0.4 (12/2521); 0.6 (18/2512); 1.8 (4/219) percent, respectively, at the dumpy locus. Thirty-two of 35 exceptions were mosaic mutations (classifiable phenotypically as half-and-half mosaics). Of these, 11 showed gonadal mosaicism, three were gonadal complete mutations, 12 were gonadal nonmutants, and six were sterile or dead. There was no evidence of gonadal mosaicism present in 250 phenotypically nonmutant F_1 males sampled from the 1.8% series. Comparison of these results with the mutational spectrum induced by X-rays (CARLSON, Genetics 44:347–373, 1959) indicated no differential production of unique dumpy phenotypes after injection of the quinacrine mustard except for a lower frequency of variegated heterochromatic dumpy mutants and of the most extreme mutant phenotype (*olv*) than is obtained with X-irradiation. These results suggest that the quinacrine induces mutations primarily in the old gene (presumably in one strand of the DNA molecule) and that these changes are of a point mutational nature, not associated with chromosomal disarrangements. If mutagenesis with chemicals in general follows the same pattern expressed here, then only the rarer whole-body mutations and those mosaics with completely affected gonads would be detected by the usual procedure followed in testing for induced lethals. This would be considerably less than the true induced-mutation frequency, and it is possible that mutagens not found to be effective in lethal tests previously may actually turn out to be potent mutagens when their ability to induce visible mutations at specific loci is determined. The results are also consistent with a theory of mutagenesis proposed by MULLER, CARLSON, and SCHALET (Genetics 46:213–226, 1961) and may explain the interesting observation of the FAHMYS that certain chemicals appear to produce a preponderance of visibles in relation to lethals (J. Genet. 54:146–164, 1956). (This research was supported by grant G15569 from the National Science Foundation and by grant Cy-4615 C1 from the National Institutes of Health.)

CHANG, TSUENG-HSING, Biology Department, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas: *Dependence of the oxygen-effect counteracting property of argon upon the temperature.*—It was reported that argon had the property of counteracting the oxygen effect when Drosophila males were given 1,000r X-rays. Further experiments were done to find out if the aforementioned property was temperature dependent.—Mixtures of 10% oxygen in argon at different total pressure were prepared at 23°C (room temperature), so that the total pressure would be down to ten atmospheres at a given temperature. *Drosophila virilis* males were treated in these mixtures for 50 minutes, with or without 1,000r X-rays, and the percentages of egg hatch were scored. The following results were obtained. At 23°C: 0r, 95.6 ± 0.7%; 1,000r, 72.1 ± 0.9%. At 15°C: 0r, 91.5 ± 0.7%; 1,000r, 66.5 ± 0.9%. At 0°C: 0r, 96.2 ± 0.7%; 1,000r, 57.2 ± 1.5%. The percentage of egg hatch decreased as the temperature, in which the sperm were treated, decreased. It is concluded that the oxygen counteracting effect of argon decreases as the temperature decreases. (Supported in part by Research Training Grant CRT-5047 from National Institutes of Health, and by U. S. Atomic Energy Commission Contract AT-[40–1]-1828.)

CHAPIN, MARGARET, and G. R. DUBES, University of Kansas School of Medicine, Kansas City, Kansas: *Tryptophane-requiring mutant of poliovirus.*—A tryptophane-response (*tr*) mutant of the Mabie strain of antigenic type 3 poliovirus was obtained by passages in monkey kidney tissue culture cells supplied with high concentration (2μm) of L-tryptophane.—When temperature is 37.0–37.5°C and *p*H 7.0–7.5, the *tr* mutant produces 10^2 times as many plaques on cells supplied with a high concentration of L-tryptophane as on cells not supplied with L-tryptophane, whereas wild type produces slightly fewer plaques on high-tryptophane cells. The lower the temperature, the lower the concentration of L-tryptophane required by the *tr* mutant; below

about 35°C no exogenous supply is needed.—Like the wild type, the *tr* mutant is L-cystine-requiring. The L-tryptophane requirement is not met by L-cystine, D-tryptophane, tryptamine, indole, L-tyrosine, DL-α-alanine, DL-serine, DL-leucine or L-histidine, but is by DL-tryptophane, DL-5-methyltryptophane or indole-3-acetic acid; anthranilic acid and DL-phenylalanine show slight activity.—Adsorption rate of *tr* mutant to cells was not accelerated by pre-inoculation incubation of cells with high L-tryptophane, by using *tr* virus produced in presence of high L-tryptophane, and by high L-tryptophane in medium during adsorption; however, rate of appearance of progeny *tr* virus was accelerated by high L-tryptophane.—Spontaneous genetic revertants to wild type were readily obtained through isolation from occasional large plaques produced by *tr* mutant on 37°C cells not supplied with tryptophane.—Virus progeny from ribonuclease-labile infective units, obtained by reacting *tr* virus with phenol, exhibited the *tr* character. Thus *tr* mutant "ribonucleic acid" carries information determining the requirement of the virus for exogenous L-tryptophane. (Aided by grant from National Foundation.)

CHEN, JANE R., M. E. SANDERS, C. J. FRANZKE, and J. G. ROSS, South Dakota State College, Brookings, South Dakota: *Genetic similarity of colchicine-induced grass-type mutants.*—The genotypes of three phenotypically similar true-breeding diploid grass-type mutants and the grain sorghum variety, Experimental 3, from which these mutants were induced by colchicine treatment, were investigated to determine their similarities. The modes of inheritance of four different contrasting characters, red *vs.* green seedling-base color, dry *vs.* juicy midrib, purple *vs.* nonpurple spotting on leaf blade, and awnless *vs.* awned, were analyzed from the results obtained from five categories of crosses. Heterosis was uniformly shown in F_1 plants of all crosses between Experimental 3 and grass-types indicating marked genetic differences between the mutants and the original variety. The uniformity between F_1 plants in each cross indicated that the mutants and the original variety are homozygous. No heterosis was shown in F_1 hybrids from the six possible crosses between the grass-type mutants indicating their genetic similarity. Single-factor Mendelian inheritance of seedling-base color and leaf spotting, and dihybrid segregation for dry midrib was found for all the grass-type mutants in F_2, backcross, and outcross progenies. The appearance of the recessive grass-type characters in F_1 plants from crosses between grass mutants suggests that the mutated genes in these mutants are identical and located at the same loci. It would seem that these mutants may be genotypically as well as phenotypically the same. The identical genotypes occurring in the mutants might possibly have arisen from the same mutagenic effect being present in each instance within the swollen coleoptile resulting from treatment with colchicine. (This investigation was supported by the Public Health Service and the National Science Foundation.)

CHOVNICK, A., A. SCHALET, and R. P. KERNAGHAN, Biological Laboratory, Cold Spring Harbor, N.Y.: *Recombination at the rosy locus in Drosophila melanogaster.*—A series of spontaneous and X-ray-induced mutants at the rosy locus (3–52.4) were subjected to recombinational analysis through the use of a crossover-selector system (WHITTINGHILL 1950). Males from a balanced stock, *In(3)MRS, M34 ry^{2} Sb/ Dfd ry^{2} Ubx e^{4}*, were mated to two series of females: (A) *M34 Dfd ry^{2} + +/ + + ry^{11} Sb Ubx* and (B) *M34 Dfd ry^{11} + +/ + + ry^{2} Sb Ubx*. Due to homozygous lethality associated with the markers *M34*, *Dfd*, *Sb*, and *Ubx*, all offspring of these crosses die except ¼ of the crossovers between *M34* and *Dfd*, ¼ of the crossovers between *Sb* and *Ubx*, and ½ of the crossovers between *Dfd* and *Sb*. Viable offspring represent only 5% of all zygotes. The remaining 95% die as larvae or pupae. From a set of such crosses most adult offspring are phenotypically rosy. The sporadic *ry*+ individuals consistently found in one of the two crosses in a set invariably carry appropriate markers indicating their origin as crossovers between separable sites within a complex locus. The double mutant types, expected in the cross that does not yield *ry*+ individuals, have not been distinguished. Three separable sites within the rosy locus have been established: (*ry^{1}, ry^{4}, ry^{8}, ry^{9}*)–*ry^{26}*–(*ry^{2}, ry^{6}, ry^{7}*). Significant failure of recombination has not been observed. Map distances, estimated as 2 × (# of *ry*+ recombinants) × 100/20 × (total surviving offspring), range from a high of 0.005 to a low of 0.0004.

An improved selective system with increased percent killing and some egg mortality has replaced the above system in current experiments.—All mutants reported here are noncomplementary for eye color and xanthine dehydrogenase activity. Current data support the existence of a single complementation group. (This investigation was supported by research grants, C-4440 and RG–7464, from the National Institutes of Health, Public Health Service, and by a grant, NSF-G6431, from the National Science Foundation.)

CLEMENT, W. M., and E. H. STANFORD, U. S. Department of Agriculture, University of Minnesota, St. Paul, Minn., and University of California, Davis, Calif.: *Chromatic and achromatic chromosome segments and their relation to chiasma frequency in alfalfa, Medicago sativa L.*—The pachytene chromosomes of alfalfa are characterized by a prominent knob on one end of each chromosome. The knobbed arms are highly chromatic, whereas the arms without knobs are largely achromatic except for the regions adjacent to the centromeres. The knobbed arms are shorter in chromosomes with submedian centromeres.—In diploid alfalfa, rod-shaped bivalents are usually formed at metaphase and ring bivalents occur infrequently. Cultivated tetraploid alfalfa, as well as induced autotetraploid alfalfa forms few multivalents at metaphase (1–3 average). Observations indicate that chiasmata frequencies are much reduced in one arm (presumably the shorter chromatic arm). This may explain the low frequency of multivalents in the tetraploid form and a predominance of rod bivalents in the diploid form of alfalfa.—If chiasma formation represents genetic crossing over a limited degree of recombination would be expected in one entire arm of each chromosome. The breeding system of this highly cross-pollinating crop may thus be more conservative than previously thought.—An alternative to the hypothesis of restricted chiasma formation in one arm is that chiasma may occur in either arm but on the average only one chiasma is formed per chromosome pair. This also would result in a rather conservative system.

COE, E. H., U. S. Department of Agriculture and University of Missouri, Columbia, Mo.: *Allelism at the C locus in maize.*—The allelic series of C^I (conventionally designated I), C, and c presents the anomaly of dominant colorless, colored, and recessive colorless expression in anthocyanin pigment synthesis in the aleurone tissue of the maize kernel. The possibility that duplication (or separable loci) might provide an explanation of the functional anomaly has been investigated through mutation and recombination analysis, with negative results. Among 2.8×10^6 C^I gametes in which mutation to C would have been detected, no valid mutations were found. However, among 2.4×10^6 C^I gametes in which mutation to c would have been detected, one mutation to c has been recovered. Mutability systems have also induced only c-like changes. A test for compoundness shows that if C^I is to be represented as compound and of the constitution $C\,I$ or $I\,C$ then the two units must be less than 0.00032 map units apart, while if C^I is of the constitution $c\,I$ the distance must be less than 0.079 units and if $I\,c$ less than 0.064 units. Consequently the inhibitor allele must be considered either as compound and $c\,I$ or $I\,c$, with the reservation that the two components are extremely close together, or as a single factor which does not mutate to one of its two known lower alleles and which has an action divergent from that of its alleles. The latter hypothesis is favored as the simpler. Bifunctional loci without duplication may provide diversity in nonessential functions.

COHN, N. S., Ohio University, Athens, Ohio: *The production of chromatid aberrations by 1,2–3,4 diepoxybutane and 2,2′ bipyridine.*—Recent investigations have shown that chromatid breaks produced by certain radiomimetic agents in combination do not interact to produce reunions. Conversely, breaks induced by X-rays will interact with those produced by a variety of chemical agents. These effects have been discussed in terms of possible differences and similarities in the bonds broken by the various agents. Several types of combined treatment were performed on the lateral roots of *Vicia faba*. The effects of a chelating agent, 2,2′ bipyridine which is specific for iron, and an alkylating agent, 1,2–3,4 diepoxybutane were studied alone and in conjunction with potassium cyanide and X-rays. On the basis of earlier investigations, the results were some-

what unexpected. When the roots are treated with diepoxybutane and potassium cyanide, the frequency of exchanges produced is indicative of complete interaction of broken ends. On the other hand, treatments with X-rays and diepoxybutane or X-rays and bipyridine indicate a purely additive effect. The effect of bipyridine is especially striking since this agent produces a low, though significant frequency of breaks, all of which are located in the heterochromatic regions of the chromosomes. Additionally, bipyridine enhances the effects of potassium cyanide and diepoxybutane with respect to chromatid exchanges. The results are presented in terms of specific chemical linkages in the chromosomes. (This work was supported in part by the Atomic Energy Commission under contract AT[11–1]826.)

Coy, D. O., and R. W. Tuveson, Wayne State University, Detroit 2, Michigan: *Heterocaryosis between presumed species of Fusarium.*—Certain diauxotrophic strains of *Fusarium oxysporum* f. *pisi* were found to form stable heterocaryons with diauxotrophic strains of *Fusarium (Hypomyces) solani* f. *cucurbitae*. The interspecies heterocaryons resembled those formed within the two species. As had been observed for heterocaryons within the species, those between the species produced spores predominantly of one genotype when grown on minimal medium. The minority nucleus was demonstrated to be present in two heterocaryons which had been grown on minimal medium supplemented with the metabolites synthesized by their respective major nuclear component. With specific heterocaryons grown on supplemented minimal medium, the minority nucleus was not detected. With certain of the diauxotrophic strains, viable interspecies heterocaryons could not be produced even though the diauxotrophic strains utilized were capable of participating in intraspecies heterocaryons.

Cribbs, Richard M., and E. Englesberg, University of Pittsburgh, Pittsburgh, Pennsylvania: *"Negative interference" from near and afar with arabinose negative mutants of Escherichia coli B/r.*—Additional arabinose negative mutants of *Escherichia coli* B/r have been ordered by three factor reciprocal crosses with bacteriophage P1, in which leucine independence is used as a nonselective marker. (Gross and Englesberg, Virology 9:314, 1959). Recent findings indicate that one of the B group mutants (arabinose-29), when crossed with some adjacent and nonadjacent mutational sites in the same gene, shows high "negative interference" (i.e., the ratio of the number of leucine independent markers among the arabinose recombinants were found to be equal in reciprocal crosses, where in one case, a double crossover and, in the other, a quadruple crossover is required). An independently isolated mutant, located at the same site as arabinose-29, does not exhibit "negative interference" with any of the B group mutants. Possible explanations of this phenomenon will be discussed.

Cromroy, Harvey L., (Introduced by J. S. Kirby-Smith), Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Modification of ultraviolet-induced aberration frequencies in Tradescantia pollen.*—The effects of a variety of pre- and posttreatments on ultraviolet-induced chromosomal aberrational frequencies in Tradescantia pollen have been investigated. The parameters studied included humidity, temperature and visible radiation. All the ultraviolet treatments were made at 50% relative humidity and with an exposure of 1×10^6 ergs/cm^2 of 2537 A. Pretreatments in a water-saturated chamber decreased frequency of both chromatid and isochromatid aberrations, whereas posttreatments in the same type of chamber caused a slight decline in chromatid breaks alone. Heat treatment of pollen (40°C for 30 min) prior to ultraviolet radiation exposure results in an increased aberration frequency; a similar post ultraviolet heat treatment decreases aberration frequencies. These effects of humidity and heat correlate with factors affecting the magnitude of the synergistic effect reported by Kirby-Smith, Nicoletti and Gwyn. In contrast to the direct dependence of this synergism on pretreatment with visible light in the wave lengths 4000 to 6000 A, the present experiments lead to a reduction in the ultraviolet aberration frequencies. Although the isochromatid aberration frequency varies in all treatments where reduction of aberrations occurs, the percentage of incomplete isochromatids remains constant. This factor would tend to rule out a simple explanation based on interference

with a chromosome-rejoining mechanism. Interpretation of results, especially visible protection appears to be compatible with a mechanism similar to that proposed for photoprotection in certain bacteria.

DALY, K., San Fernando Valley State College. Northridge, California: ***The effect of fast neutrons on quantitative variability in Arabidopsis thaliana.***—A study to determine the effect of fast neutrons in the induction of quantitative variability was initiated utilizing flowering time in *Arabidopsis thaliana* (L.) Heynh. (Cruciferae). Seed of the race 'Estland,' stored prior to irradiation over dry $CaCl_2$ at room temperature, were exposed to 3760, 9400, 12000, 14000 rads and hydrated immediately. The date of flowering was recorded in first and second generations following irradiation and transformed to common logarithms for analysis.—The effect of seed exposure was evidenced by a change in the mean in the first generation, the number of days to flowering being greater with increasing dose. The subsequent N_2 generation was obtained from random samples of seed produced by the N_1 generation from self-pollination. Both means and variances of the four N_2 populations were significantly greater than those of the nonirradiated population. The induced genetic variation was estimated in each irradiated population by partitioning the within-plot variance. The regression of induced variance on dose did not deviate significantly from linearity. (This investigation was carried out during the tenure of a Postdoctoral Fellowship [CF-8248] from the National Cancer Institute, U. S. Public Health Service, while at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

DHILLON, T. S., and E. D. GARBER, University of Chicago, Chicago, Ill.: ***Transmission of supernumerary chromosomes in Collinsia.***—Supernumerary chromosomes were found in hybrids between *C. sparsiflora* subsp. *arvensis* and *C. bruceae* and in *C. solitaria.* The number of such chromosomes in progeny from crosses between plants with and without supernumeraries indicated that these chromosomes had undergone nondisjunction on the male and female side in *C. sparsiflora* subsp. *arvensis* × *C. bruceae* and on the female side in *C. solitaria.* Plants with 1–4 supernumeraries were fertile; plants with 5–8 supernumeraries were sterile. (Supported by grants from the National Science Foundation and the Abbott Memorial Fund, University of Chicago.)

DIVELBISS, J. E., State University of Iowa, Iowa City, Iowa: ***Structural complexity of the brown locus in Drosophila melanogaster.***—The brown locus (*bw*, 2–104.5) was found to be divisible into two subunits in recombination studies using the four alleles: bw, bw^{81}, bw^{75} and bw^{59}. Plexus (*px*, 100.5) and speck^2 (sp^2, 107.0) were used as outside markers. In all crosses *In(2L)Cy* and in all but one cross $In(3LR)Ubx^{130}$ were used to enhance crossing over. A total of six wild type and two double mutant recombinant chromosomes were recovered. The alleles bw and bw^{75} occupy a site to the left of bw^{59} and bw^{81} with approximately 0.001% recombination between the subloci. An additional 15 nonrecombinant $bw+$ chromosomes were recovered. These reversions are not due to reverse mutation, and it is unlikely that they could all be explained by double crossing over. The reversions are probably due to an event similar to the "gene conversion" phenomena found in microorganisms. Although bw^{59} and bw^{81} have not been separated by crossing over, reversions were obtained from the bw^{59}/bw^{81} heterozygote. The recovery of these reversions indicates that the alleles occupy different mutational sites. In one case five reversions were recovered from the same culture. This suggests that they represent a cluster, which means that the reversions must come from a premeiotic event and are thus due to a mechanism other than meiotic crossing over. Fluorometric determinations of some of the pteridines in the eyes of the homozygotes and all possible heterozygotes gave no indication of complementation between alleles. Thus, these alleles all belong to the same functional unit. (Supported by a co-operative graduate fellowship from the National Science Foundation.)

DORIA, GINO, (Introduced by R. F. KIMBALL), Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: ***A quantitative bio-assay for homograft reaction.***—A method has been

devised to study the kinetics of homograft reaction by isolated lymphoid cells.—Spleen cells from F_1 hybrid mice sensitized against rat RBC are transplanted into lethally irradiated parental strain (P_1) mice, which also receive the test antigen. Antirat RBC agglutinins produced in the serum of the irradiated recipients are due to the secondary response of the transferred spleen cells, and the $\log_2$ of the titer is a linear function of the $\log_2$ of the cell dose. If the recipients are also injected with isologous (P_1) lymphoid cells from mice preimmunized against the other parental strain (P_2), the agglutinin production by the F_1 spleen cells is impaired. The reduction in agglutinin titer depends on the number of P_1 cells injected and on their immunologic effectiveness against the F_1 cells. Hence, for any given dose of P_1 cells it is possible to establish the percentage of agglutinin producing F_1 cells that are inactivated or rejected. Since the F_1 cells are the only possible target for the immunologic activity of the P_1 cells, this technique differs from others (Celada, *et al.*, Federation Proc. 20:33, 1961), in which F_1 mice are the irradiated recipients and one of the parental donors is sensitized against the test antigen.—Among the immunogenetic applications of this method, studies of homotransplantation immunity in chimeras will be discussed.

Dorn, G. L., and A. B. Burdick, Purdue University, Lafayette, Ind.: *Fine genetic analysis of the miniature-dusky complex in Drosophila melanogaster.*—Transheterozygotes containing all possible combinations of the mutants m^{59a}, m^{57i}, m^{D} (a dominant mutant), *dy*, dy^{58k}, and Df(1)259–4 and certain combinations of m^{60l} and dy^{61a} were produced and the progeny of these combinations were scored for wild-type recombinants. The double and single mutants are probably indistinguishable. Df(1)259–4 uncovers the miniature but not the dusky mutants. Each of the transheterozygotes was marked on the left with vermilion (*v*) and on the right with garnet (*g*). 1,766,872 flies were scored and 89 wild types found. Complementation studies were performed on each of the genetic combinations and the differences in the amount of wild-type restoration were determined.—The total length of the recombination map is 0.04 units. The mutants fall into a linear recombination arrangement. All recombinants were of the "classical" (reciprocal) type with respect to the outside markers except those involving Df(1)259–4 and the miniatures, which appeared to show "nonclassical" recombination.—The mutants can also be arranged in a linear fashion on the basis of the degree of wild-type restoration (complementation) produced by any two mutants. The order of the elements on the complementation map coincides with the order on the recombination map.—These results seem to indicate that the miniature and dusky mutants comprise one functional continuum which is subdivisible by recombination and graduated by complementation in a manner directly related to distance.

Doudney, C. O., Tsuneo Kada, and F. L. Haas, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas: *Alternate mechanisms of deoxyribonucleic acid replication and mutation induction by radiation in bacteria.*—Recent studies have established that postirradiation treatments blocking RNA and protein formation lower markedly ultraviolet induced mutation frequency in bacteria. Comparable results for a portion of X-ray-induced mutation have been found. The potential mutation is unstable in the cell until the RNA and protein formation necessary to recovery of the UV damaged DNA synthetic system occurs.—If certain auxotrophic strains of *Escherichia coli* are exposed to a given dose of X-ray or UV and then allowed to incubate for increasing periods of time prior to a second irradiation, an increasing mutation frequency response is observed upon plating on minimal medium limiting to formation of this capacity. The increased response is lost upon the initiation of postirradiation DNA synthesis. The data suggest that following X-ray or UV exposure and RNA-protein structure intermediate in DNA replication is formed which results in high mutation response when subject to a second irradiation.—It is proposed that UV induced mutation is the consequence of (1) the induction of a block to DNA synthesis via the Kornberg enzyme system by a photochemical effect of UV which binds the two complementary strands of DNA together to prevent replication, (2) the resultant adoption of an alternate mechanism of DNA replication based on passage of information from the double stranded DNA to an RNA-protein intermediate

and back to daughter DNA, and (3) the incorporation of UV modified purine or pyrimidine precursors into the RNA-protein resulting in DNA modified by a nucleotide pair unit. Physiological and biochemical evidence bearing on this hypothesis will be presented.

DOWNS, WM. G., JR., DAVID FITZPATRICK, JAMES R. NEELEY, and ALTA FAYE JOHNSON, Tennessee Polytechnic Institute, Cookeville, Tennessee: *Differences in response to irradiation of different strains of rats.*—Although everyone in the field seems aware that there are marked "strain differences" in response to stress of all sorts, results obtained by us indicate that the difference in response to irradiation on the part of mammals is greater than has been generally believed.—In the course of our studies on the effects of irradiation on the leucocytogenic organs and the endocrines, (including 100 or more animals of each breed), we have found that an LD 50/30 dose of gamma rays varies from 800r for a Sprague-Dawley strain of albino rats, to 1100r for our "Tec" hybrid strain of albinos. 1200r does not seem to be an LD 50/30 dose for the agouti-hooded strain of Long-Evans rats, a start of which was obtained from their laboratory in California. It is highly possible that a black-hooded strain requires even higher doses. A difference of 50% seems quite remarkable and would suggest that the response of humans to irradiation might be greatly influenced by racial background.

DUVICK, DONALD N., Pioneer Hi-Bred Corn Company, Johnston, Iowa: *Genetics of partial fertility restoration of "Texas" cytoplasmic pollen sterility in maize.*—Of four partial fertility restorer (PFR) inbred lines tested to date, each apparently owes its partial restoration to a single dominant gene although superficially the partial restoration of each has appeared to be controlled by many genes. The demonstration of a single PFR gene in these lines is dependent upon the presence of a particular genetic background and proper environmental conditions during the pollen shedding period. Allelism tests between these four lines have not yet been made.

EHLING, U. H., and M. L. RANDOLPH, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Skeletal abnormalities in offspring of irradiated male mice.*—Over-all genetic damage from radiation, which cannot be estimated accurately from mutational information in a limited number of genes, must be measured empirically. In a series of experiments, skeletal damage was measured in the offspring after irradiation of either spermatogonial or postspermatogonial stages with 600 rads of X-rays, 200 rads of monoenergetic 14 Mev neutrons, or 80 rads of monoenergetic 3 Mev neutrons. Irradiated 101 males were mated to C3H females. Litters were sacrificed at the age of 26–28 days. Whole skeletons were examined in detail in Alizarin red preparations.—Abnormalities were tabulated either as: Class 1, abnormalities that were observed only once in the whole experiment; or as Class 2, abnormalities that were found in two or more animals. Pooling the present results from all experiments showed no difference in the frequencies of Class 2 abnormalities derived from irradiated and nonirradiated males. The frequency of Class 1 abnormalities in the two groups differs significantly (22 out of 443 offspring from irradiated males and seven out of 525 offspring from control males, $P = 0.0018$). The distinct increase of abnormalities in offspring from irradiated males demonstrates that skeletal effects are suitable for measuring part of the over-all damage from presumed dominant mutations. More data are being collected to evaluate the response of different gametogenic stages.

EISENSTARK, A., and N. D. REED, Kanasas State University, Manhattan, Kansas: *Mutation affecting head antigen of phage T3 of Escherichia coli.*—Mutation affecting the neutralizable antigen of phage T3 was described previously (EISENSTARK, MAALØE and BIRCH-ANDERSON, Bacteriol. Proc. 1960, page 81). Antibodies against one of the T3 phages (T3B), are incapable of neutralizing another T3 strain (T3C). Anti-T3C sera, however, can inactivate both T3B and T3C. Also, the tail structure is different in these two strains: T3C has a long flexible tail, in contrast to T3B which has a short tail.—In the course of density determinations of these variants by the CsCl gradient technique, certain results led us to suspect that mutation of T3B to T3C affects a change in head antigen as well as neutralizable antigen. When anti-T3B serum is added

to phage T3B, the density is lowered from about 1.49 to below 1.44. However, if anti-T3B serum is added to T3C, no density change is detected. In cases where plaque-forming ability of phage would be destroyed by antiserum, phage were labeled with p32 in order to detect the position of phage in the centrifuge tube.—If it is assumed that the neutralizable (tail) antigen and the head antigen are different, as in T-even and lambda phage particles, then it is concluded that the mutation of T3B to T3C affects changes in both of these antigens. (This work supported by National Science Foundation, Grant No. G12332.)

EMERY, GEORGE C., and JAMES L. BREWBAKER, Brookhaven National Laboratory, Upton, N.Y.: *The cytogenetics of the incompatibility locus in Petunia inflata.*—Primary trisomics, including the trisomic for the *S* chromosome, have been synthesized in *Petunia inflata* and studied in advanced progenies. The trisomics for the *S* chromosome were readily distinguishable by self-fertility, increased pollen sterility, and large flower size. Trisomics for this chromosome made possible its karyotypic identification. Trisomics with three different *S* alleles were isolated and used to determine the linkage of the *S* locus to centromere by the double reduction method.—Numerous *S* mutants in *Petunia inflata* have been studied. The majority of self-fertile mutants arising from X-irradiation have resulted from the presence of an added centric fragment. The inheritance and cytology of *S* allele centric fragments will be reported. Meiotic pairing has been observed between the trisomic *S* chromosome and the centric fragment in plants containing both. The proximity of the *S* locus to the centromere similarly was inferred by karyotypic studies of the centric fragments. The significance of centric fragments and their relationship of *B* chromosomes will be discussed.

ENGLERT, DUWAYNE C., and A. E. BELL, Population Genetics Institute, Purdue University, Lafayette, Indiana: *Genetic differences in the growth curve of Tribolium castaneum Herbst.*—Growth of two inbred lines (C_1 and Va) of *Tribolium castaneum* and their reciprocal hybrids was investigated by observing body weight daily for all developmental stages from egg to adult. Variables given specific consideration were 14-day larval weight, first-day pupal weight, adult weight and pupation time. Fourteen-day larval weight, first-day pupal weight and adult weight were positively correlated with one another. Values of +.51 and +.65 were obtained for the correlations of first-day pupal weight and adult weight with 14-day larval weight; the correlation of first-day pupal weight with adult weight was +.94. Pupation time was negatively correlated with each of the other traits with values ranging from — .37 to — .87. All correlations were found to be significant. Larval growth to 14 days of age was also considered and a logarithmic transformation of daily weights resulted in a linear response.—The hybrids exhibited heterosis for all variables measured (including the linear regression coefficients of the larval growth period of interest) when compared to the average of the parental lines. Of the variables considered, 14-day larval weight exhibited the highest percent of heterosis (82 percent). (Work supported by a grant from the National Science Foundation).

EVANS, J. E., University of Houston, Houston 4, Texas: *Regulation of growth in Proteus vulgaris by DNA extracts.*—The complexity of strain specific transformation by DNA extracts is associated with the production of a multichromatinic bodied cell in *P. vulgaris.*—This multichromatinic bodied cell develops directly from a "short form" to a "swarmer" in the absence of cell division and is characterized by the simultaneous division of chromatinic material.—Here the interaction between DNA extracts and the parent cells has been shown to regulate cell division, growth, and the development of *Proteus vulgaris.*

FORSTHOEFEL, PAULINUS F., and ANNA MARIE KUHARCIK, University of Detroit, Mich.: *The problem of anemia in mice homozygous for Strong's luxoid gene.*—Both in the strain of origin and in a strain produced by repeated backcrossing of carriers for Strong's luxoid gene to pure strain C57BL/10 mice, some homozygous mice (*lst/lst*) appear anemic at birth. Counts of the numbers of red blood cells, determinations of hematocrits and hemoglobin contents made on

newborn homozygous luxoid mice and on their normal and polydactylous carrier sibs as controls have shown that most of the luxoid homozygotes have a normal blood picture but that about a third have significantly low red blood cell counts and other indications of a low grade anemia. Study of the blood of 16 litters dissected out shortly before birth has shown no signs of anemia in any of the homozygotes. Therefore the anemia develops only after birth.—The umbilicus is much closer to the genital tubercle (distance 0.5–1.5 mm) in newborn Strong luxoid homozygotes than in their normal or carrier sibs (distance 3.5–6 mm). More significantly, a strip of skin is frequently missing between the umbilicus and the genital tubercle in the homozygotes but never in the normal or carrier sibs. Also the body wall in this region is very weak in the homozygotes and occasionally ruptures. These observations have led to the hypothesis that the anemia is the result of excessive bleeding produced by some damage to the arteries in the umbilicus region at the time of birth. In some cases this prolonged bleeding has been actually observed. (Supported by Grant C-3613 from the National Institutes of Health.)

FOSTER, M., and LUCINDA THOMSON, University of Michigan, Ann Arbor, Mich.: *Allelic relations at the brown locus of the house mouse.*—Several melanogenic attributes have been studied using skins of the four homozygotes and six allelic compounds involving the alleles "Light" (B^{LT}), Black (B), Cordovan (b^c) and brown (b), principally on intense, self-colored (aa) backgrounds. The attributes include tyrosinase and dopa oxidase activities (measured manometrically), as well as turbidimetrically measured (1) natural melanin content, (2) newly formed melanin within incubated skin subsamples and (3) darkening of incubation media due to escape of melanogenic intermediates from incubated skin.—All ten genotypes can be recognized from their characteristic numerical profiles obtained from comparisons for each measured attribute. Moreover, depending upon the phenotypic attribute used for genotypic comparison, the physiological relations of alleles in the various compounds range from incomplete dominance (noncomplementary interaction) through complete dominance to possible cases of heterotic interaction (luxuriance), the last usually involving the presence of b.—Although the role of residual genotype has not been adequately assessed, these observations appear consistent with the view that the genetic region occupied by these alleles is complex and that this locus is in one or more ways concerned with controlling the environment in which tyrosinase acts. (Supported in part by NIH Grant C-4305 and NSF Grant G-6480.)

FRIEDMAN, LAWRENCE D., University of Wisconsin, Madison, Wis.: *X-ray induced viability mutants in Drosophila melanogaster.*—In order to determine the relative importance of lethal *vs.* detrimental mutations Muller-5 (Basc) and wild type males segregating from the same culture were irradiated with 1000, 3500, and 7000r. These were mated with Basc/+ females from the same culture, and the F_1 heterozygous females mated individually with asc males which are the same as Basc except for the absence of Bar. The F_2 male progeny were counted, and the ratio of + to Basc where the Basc chromosome had been irradiated was compared with those where the + had been irradiated. The lethal frequency was .029/1000r for the Basc chromosome and .020/1000r for Canton-S, a significant difference.—Early studies by TIMOFFEEF-RESSOVSKY and KERKIS showed a high fraction of the total genetic load to be due to detrimentals, whereas the more recent work of KAFER showed a smaller fraction. Our results are in close agreement with KAFER, the ratio of the detrimental to the lethal load being 0.125. In our studies a lethal was defined as having less than 10 percent of the normal viability. The results indicate that either very few detrimental mutations are induced or that individually they have very minor effects. (Work supported by grant No. RG7666 from the National Institutes of Health, U. S. Public Health Service.)

FRYE, SARA H., 701 Atwater, Bloomington, Indiana: *Structure of "yellow" mutations induced by X-rays of scute^8 chromosomes of different germ cells in Drosophila melanogaster.*—577 "yellow" mutations induced by X-rays in scute^8 chromosomes of different germ cells were genetically analyzed to determine their qualitative structure. Order of the loci is assumed to be

lJ1, *y*, *ac*, and *bb*. The symbol + indicates the normal allele while — indicates an affected or deficient locus, the locus involved being indicated by the position of the symbol. An additional lethal (if present) somewhere to the right of *bb* is symbolized as — in the fifth position. The following are structural frequencies of the "yellow" mutants X-ray induced in oogonia, oocytes, and mature sperm, respectively: — — — + 4, — — — + — 1 (total 5); — — — + 14, — — — — 5, — — — — — 1, — — — + — 3, + — — + 5, + — + + 3, + — — + — 3, + — — — — 1 (total 35); — — — + 374, — — — — 43, — — — — — 31, — — — + — 16, + — — + 41, + — + + 22, + — — + — 4, + — + + — 1, + — — — 2, + — — — — 3 (total 537).—All — — (±) (±) chromosomes of oogonia and oocyte "yellows" were tested for translocations involving the fourth chromosome (*Cat*+). None of the oogonia "yellows" were capped by *Cat*+ (IVR). Two of the oocyte "yellows" (symbolized — — — —) were capped by *Cat*+ (IVR). Mature sperm "yellows" were tested for the second (IIL, IIR) and fourth chromosome tips. Twenty-two cases were found. Tips from IIR (11/22 exceeded all other autosomal tips detected. All yellow half-translocations were *deficiency half-translocations.*—The structure of breakage "yellows" in scute^8 chromosomes is not dependent upon either the X-ray dose (even ten spontaneous "yellows" were classifiable as either minute or gross structural changes) or the germ cell stage.—The means by which X-ray induced (and possibly spontaneous) breakage and intragenic mutation can be separated is still not available. A consideration of the frequency, functional and structural types of chromosome breaks and their interactions may yield a more realistic and unifying approach to transmissible changes (both forward and back) than the concept of intragenic mutation.

FUERST, R., and BARBARA J. BEDAIR, Department of Biology, Texas Woman's University, Denton, Texas: *Adaptation to chemical inhibitors and the induction of fragmentation mutants in Neurospora.*—The growth of *Neurospora crassa* Em5256A may be inhibited by the addition to the medium of certain antimetabolites, some of which are also antineoplastic agents. Attempts were made to adapt the organism to ever increasing doses of some of these chemical inhibitors. This was done using the plate well method. It was found possible to adapt Neurospora to normally lethal concentrations of 8-azaguanine, amethopterin, aminopterin, 4-aminopyrazolo (3,4-d) pyrimidine, 6-diazo-5-oxy-nor-L-leucine, 2,6-diamino-purine, and others. Upon prolonged storage of resistant strains an apparent reversion to sensitivity to the drugs was observed. This is attributed to the formation of heterocaryons between resistant and reverted sensitive nuclei. For this reason sexual crosses of the previously adapted strains with the unadapted wild type KJTa were set up. The method used was essentially the spore shooting technique of NEWMEYER. Offsprings expected were inhibitor resistant strains, wild type, and perhaps some biochemical and morphological mutants due to exposure to inhibitors being potential mutagens. Offsprings actually obtained were of course wild types, but also an unexpected type of mutant that may be designated as "fragmentation mutant." These fragmentation mutants were obtained in high proportions among the progeny from crosses of the unadapted wild type with 6-diazo-5-oxy-nor-L-leucine adapted strains and also from crosses with DL-thioctic acid adapted strains.

FUSCALDO, KATHRYN E., and ALLEN S. FOX, St. John's University, Jamaica, N.Y., and Michigan State University, E. Lansing, Mich.: *An immunogenetic analysis of white-variegated position effects in D. melanogaster.*—The antigenic specificities of several stocks of *D. melanogaster* were analyzed by means of agar-diffusion techniques. Stocks possessing euchromatic-heterochromatic rearrangements involving the white pseudoallelic loci were tested along with the mutants *W*, *W^e*, *W^{az}*, *bwcn* and a wild isogenic stock, Oregon-R-I.—In all cases an alteration of the relationship of heterochromatin to the white loci resulted in a change in the form of an antigen, designated H(w)-1. The antigen H(w)-1 exhibits a higher combining power in the inverted and translocation stocks than in the wild or mutant stocks. The difference is most probably associated with a difference in the configuration of the antigenic site as well as with a difference in the number of combining sites. The effect is reminiscent of the effect of the Y chromosome on the antigen Y-1 (Fox 1959).—This change in the immunological properties of an antigen without

a concomitant change in its specificity is associated with the disturbance of the normal euchromatic-heterochromatic balance in the cell. Data will also be presented on the effect of the heterochromatic Y chromosome on the antigen H(w)-1.— These results along with those of Fox (1959) suggest a role for the heterochromatic segments of the genome in specifying the tertiary structure of a protein. The primary structure, that is the sequence of amino acids is thought to be controlled by the individual euchromatic loci through an RNA intermediary.—(Supported by grant C-2440 from the National Institutes of Health, U. S. Public Health Service.)

GALL, J. G., and H. G. CALLAN, University of Minnesota, Minneapolis, Minn., and the University, St. Andrews, Scotland: *Patterns of synthesis in lampbrush chromosome loops.*—H^3-uridine and H^3-phenylalanine have been used to study the synthesis of RNA and protein in individual lateral loops of lampbrush chromosomes from the newt, *Triturus cristatus*. Each loop consists of a ribonucleoprotein matrix surrounding a submicroscopic axis which contains DNA. Loops are asymmetric in the sense that the matrix is more abundant at one end (the thick insertion) than at the other (the thin insertion). The majority of loops become uniformly labeled after a single subcutaneous injection of H^3-uridine. Some loops, including the so-called "giant granular loops" on chromosome XII to which particular attention has been paid, show a different pattern. One day after the H^3-uridine injection the giant granular loops are *unlabeled* except for a short region at their thin insertion. During the succeeding ten days the label spreads around the loops, so that at the end of this time they are uniformly radioactive. The incorporation of H^3-phenylalanine in the giant granular loops (and possibly in all other loops as well) does not follow this sequential pattern. The giant granular loops are uniformly labeled one day after injection of the radioactive amino acid. To account for the sequential labeling with uridine we postulate that the loop is not a stationary structure, but rather is moving like a conveyor belt. Since the size of a loop does not change within the time limits of this type of experiment, we further postulate that the loop axis is being spun out at the thin insertion and is winding up again at the thick end. RNA is synthesized only at the thin insertion, is slowly carried around the loop, and is destroyed or shed into the nuclear sap at the thick insertion. This model will be discussed in relation to questions of gene structure and function.

GARBER, E. D., and T. S. DHILLON, University of Chicago, Chicago, Ill.: *A cytogenetic study of reciprocal translocations induced by ionizing radiation in Collinsia heterophylla.*—Four reciprocal translocations involving two non-homologous and one involving three non-homologous chromosomes were obtained from dry seed or seedlings irradiated with X-rays or gamma rays. The interchange complexes at metaphase I displayed a directed orientation. Plants with an interchange complex of four chromosomes had 50–84% seed set, depending on the reciprocal translocation, and plants with an interchange complex of six chromosomes, 36% seed set. The frequency of plants with an interchange complex in progenies depended on the particular reciprocal translocation and the type of cross. Five of the seven chromosomes in the complement were involved in the reciprocal translocations. (Supported by grants from the National Science Foundation and the Abbott Memorial Fund, University of Chicago.)

GARDNER, C. O., and J. H. LONNQUIST, University of Nebraska, Lincoln, Nebraska: *Effect of linkage on genetic variances and estimates of average degree of dominance in corn.*—The F_2 generation of a cross between homozygous lines has been used to obtain estimates of additive genetic and dominance variances in corn and these estimates have been used to get some idea as to the average level of dominance of genes controlling quantitative characters. The early reports indicated the existence of overdominance, but pointed out that repulsion phase linkage could provide sufficient upward bias in the estimates of dominance variance to result in indications of overdominance even though only partial or complete dominance actually exists at the individual locus level. More recent work has supported this hypothesis. A cross between two homozygous lines of corn was advanced by random mating each generation to the F_{16}. Randomly chosen plants of the F_2, F_4, F_8 and F_{16} generations were backcrossed to each parent line and

estimates of dominance variance, additive genetic variance, and average degree of dominance were obtained for each generation. In the case of grain yield estimates of dominance variance decreased with the advance in generations and estimates of additive genetic variance increased. The estimate of average degree of dominance, which was in the overdominance range in the F_2 generation, declined rapidly with the advance in generations and appeared to be leveling off in the partial dominance range. While overdominance may exist at some loci, partial to complete dominance is believed to prevail at most loci. Overdominance is not believed to be an important factor in yield heterosis in corn.

GOWEN, MARIE S., and JOHN W. GOWEN, Iowa State University, Ames, Iowa: *Aging as related to progressive body cell mosaicism.*—Until recently it has been customary to think of an individual of a species as built up of cells of the same genetic consitution which remained unchanging in their genetic endowments throughout life. Genetic progress has furnished, until now largely in Drosophila, tracer genes to show that mosaics of cells of genetically different functions can occur within the same organism, organ or lesser tissue. How frequently these changes occur in the unmarked cells of the organism throughout development and cell maintenance is not known, but there is evidence to think such changes may have fairly high frequencies that increase with age, the incompatibilities of the cell mosaics thus formed being factors in the declining viabilities associated with senescence. Data on these effects, both as chromosome balances and genic changes, were obtained by us earlier, 1931, 1934. Recently further opportunity to test the effects of mosaicism on life span have arisen through utilizing such genes as w^{vc}. HANNAH (1955) has shown that with aging mothers there is an increase in the frequencies with which mosaics occur in their young adult progenies. HINTON's research (1955, 1957, 1959) has shown that mosaic engendering genotypes result in high frequencies of deaths in the embryonic period. Cytological examination shows high frequencies of abnormal mitosis in the unstable ring or rod genotypes. The effects of the resulting mosaicisms in the adult phases on aging as measured by life spans has been studied by us. Life spans were determined on about 200 flies of each of five different genotypes: mosaic females; not visibly mosaic females; yw^{vc} males from this strain, and males and females of Oregon E, a strain recently made homozygous for chromosomes 1, 2 and 3. These results showed that mosaicism has detrimental effects earlier than for organisms without obvious signs of mosaicism at the emergence of the imago. Radiation effects have parallel features. (This work has received assistance from Contract AT[11–1]107 from the Atomic Energy Commission. Journal Paper No. J-4114 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1180, 1187.)

GROSS, S. R., and H. S. GROSS, Duke University, Durham, North Carolina: *Some features of complementation at the leucine-4 locus of Neurospora.*—The leucine (*leu*)-4 locus of Neurospora, which has not been previously described, is located approximately one unit distal to the mating type locus and about 15 units proximal to *leu-3* in the left arm of Linkage Group I. A functional analysis of 118 mutants obtained by the inositolless death enrichment procedure after ultraviolet irradiation yielded the following information: (1) Forty-nine of the mutants complemented one or more mutants. (2) Of the 49 complementing mutants, eighteen different classes were observed which could be arranged in the usual linear overlapping display so that at least 11 complementation units could be deduced. (3) Of the 11 complementation units, only four are defined by mutants whose mutational alteration is functionally confined to a single complementation unit. (4) The functional alteration of 34 of the complementing mutants spanned two or less complementation units. These mutants are clustered in about equal frequency at both ends of the complementation map. (5) Most of the mutants in the clusters can easily be distinguished from each other by their degree of leakiness, efficiency of complementation, growth characteristics, and reversion rate. Thus far no extensive mutational "hot spots" have been observed. The majority of the complementation units in this locus as well as others are determined by the unique behavior of specific mutants. In this locus, as in others previously reported, very few mutants are obtained which do not functionally overlap regions of clustering.—From these data

and from an analysis of other published complementation maps, it can be concluded that the number of functional areas involved in complementation may be defined by the number of clusters observed in the complementation map. It can therefore be proposed, among other possibilities, that complementation of the protein determined by the *leu-4* locus involves polymerization of at least two identical but singly inactive polypeptide chains in such a way that proper polymerization depends upon the structure of two sensitive areas of the monomer. Successful complementation depends upon the structural configuration within the sensitive areas and the structural and configurational relationships of the areas to each other. A more extensive discussion of this hypothesis will be presented as well as further information pertinent to the de-emphasis of the importance of the complementation unit and emphasizing clustering in relation to function. (This work was supported in part by United States Public Health Service Grant No. RG-7250 and American Cancer Society Grant No. E-238.)

HANSEN, AFTON M., and ELDON J. GARDNER, Utah State University, Logan, Utah: *A new second chromosome eye mutant in Drosophila melanogaster expressed only at high temperatures.*—A stock of wild flies consistently produced offspring with abnormal eyes when reared at 30°C. The controls at 25°C had normal eyes. Results of experiments indicated that the eye abnormality is controlled by a recessive gene located on the second chromosome. A temperature of 30°C is necessary for maximum penetrance. The temperature effective period for eggs beginning development at 30°C is from 42 hours to 68 hours after egg deposition. The entire 26-hour period is necessary for maximum penetrance.—This mutant overlaps the wild type completely at 25°C and shows incomplete penetrance and variable expressivity. Either or both eyes may be affected. The abnormality involves the lower half of the eye. Expressions vary from a flattening of the ventral portion of the eye to a slight horizontal indentation across the lower one third of the eye. In some cases tufts of vibrissae are present on the anterio-ventral border. Penetrance is greater in males than females. Further studies are being conducted to identify the locus of this mutant in the second chromosome.

HECHT, A., Washington State University, Pullman, Wash.: *Partial reduction of an incompatibility substance in the styles of Oenothera organensis.*—Styles of *Oenothera organensis* were excised from field-grown plants late during the afternoon just prior to the time of anthesis. Their stigmas were either self-pollinated or pollinated with genetically compatible pollen of this species, and the styles were placed in large Petri dishes on moist filter paper. After about 16 hours at a constant temperature of 27°C, the styles were crushed, then treated in an alcoholic iodine solution to stain the pollen tubes; the lengths of any tubes from the base of the stigma were measured by examining these preparations under a dissecting-binocular microscope.—In all self-pollinated styles, no pollen tubes were observed in the stylar tissues, and very few had penetrated even into the superficial tissues of the stigma. Where compatible pollen was used, a large number of tubes were seen to have penetrated more than 100 mm from the base of the stigmas during the same time interval.—When styles later self-pollinated were first treated for five minutes in water at 50°–57°C, a considerable penetration of pollen tubes into the stigmatic and stylar tissues was obtained: many tubes grew to more than 35 mm from the base of the stigma. These results are tentatively explained as being due to an inactivation of an incompatibility substance or precursor as the result of the treatment in warm water. (Supported by Washington State Medical and Biological Research funds and by the National Science Foundation.)

HENNEN, SALLY, (Introduced by ROBERT BRIGGS), Indiana University, Bloomington, Ind.: *On the capacity of Rana pipiens nuclei to promote development in their own cytoplasm after replicating in Rana sylvatica cytoplasm.*—MOORE (Exptl. Cell Research 1958; Develop. Biol. 1960) has shown that after repeated mitoses in *Rana sylvatica* cytoplasm, haploid and diploid nuclei of *Rana pipiens* become changed such that, in the majority of cases, they are no longer capable of promoting development beyond gastrulation when transferred back into *pipiens*

cytoplasm. Using this experimental scheme, we have transplanted *diploid pipiens* nuclei into enucleated *sylvatica* eggs, allowed recipients to develop to mid-blastulae, and transferred their nuclei back into enucleated *pipiens* eggs. Fifty-seven completely cleaved blastulae obtained from 179 back-transfer operations developed as follows: 20(35%) arrested in blastulae or gastrulae stages, 23(40%) arrested as abnormal neurulae and early tailbuds, 13(23%) formed abnormal larvae and late tailbuds, and one(2%) developed normally.—Chromosomes of the one normal and of 20 abnormal back-transfer embryos were analyzed in squash preparations. The normal animal had a normal diploid complement. All 20 abnormal embryos displayed varying degrees of aneuploidy and morphologically abnormal chromosomes such as rings and minutes.—Single and serial control transfers of diploid *pipiens* nuclei into enucleated *pipiens* eggs gave 41 complete blastulae of which 28(68%) developed into normal larvae.—These results demonstrate that the majority of diploid *pipiens* nuclei, when returned to *pipiens* cytoplasm after replicating in *sylvatica* cytoplasm, retained the capacity to promote postgastrula development. With one exception, this development was deficient. In all cases analyzed, embryos which developed abnormally contained abnormal chromosome complements. The origin of these chromosomal abnormalities remains to be determined. (This investigation was supported by research grant RG5850 awarded to Indiana University by the Research Grants Division of the U.S. Public Health Service.)

Herskowitz, Irwin H., Saint Louis University, St. Louis, Mo.: *The hypothesis of nucleotide sharing by adjacent functional units of DNA.*—The uninterrupted sequence of nucleotides in chromosomal DNA is sufficiently long to contain more than one functional unit. Moreover, previously nonadjacent nucleotides may become adjacent by crossing over, copy-choice replication, chromosomal breakage, and single nucleotide mutation. Accordingly, the possibility exists that some adjacent functional units of DNA may share nucleotides.—Heterochromatin may be composed essentially of polynucleotides of $\frac{\text{AAA}\ldots\text{A}}{\text{TTT}\ldots\text{T}}$ (in many animals) or $\frac{\text{CCC}\ldots\text{C}}{\text{GGG}\ldots\text{G}}$ (especially in CG-rich organisms.) A short (triplet?) sequence of nucleotides within either or both of these heterochromatic arrays probably specifies a simple functional product. Because of the base pair repetition, nucleotide sharing should be common in heterochromatic functioning. Where heterochromatin and euchromatin are adjacent one would also expect sometimes to find nucleotide-sharing. On this basis at least a part of each of the following phenomena is explainable as a consequence of nucleotide sharing: heterochromatic position effects (their spread and suppression), *cis-trans* position effects, certain neomorphic and "leaky" mutations, operons and adaptive enzyme formation, and certain aspects of differentiation and development. —The F particle of *E. coli*, lambda phage, centromeric DNA, centriole, kinetosome, and factors like Modulator and Dissociator are similar, or homologous, in showing present or past evidence of being episomal in nature. Presumably each is euchromatic and each is capable, when nucleotide sharing in the absence of extensive adjacent heterochromatin, of self-motility and of breaking nearby regions of DNA.—Though all of the subsidiary hypotheses may be wrong in part, and all specific explanations wrong in detail, it is suggested that nucleotide sharing may play a prime role in a large variety of biological phenomena. (Supported by Grant C4228 from the National Cancer Institute, U. S. Public Health Service.)

Hexter, W. M., Amherst College, Amherst, Mass.: *The classicality of the singed locus in D. melanogaster.*—Utilizing the singed pseudoalleles, *sn*^{3}, *sn*^{2} and *sn*, linked in the order given, the following three experiments have been designed to test for negative interference and/or gene conversion at the singed locus. (1) *sn*^{2} *oc ptg/cm ct*^{6} *sn*^{3} *sn*, (2) *sn*^{3} *oc/cm ct*^{6} *sn*^{2} *sn* and *sn*^{3} *oc/cm ct*^{6} *sn*^{2}, (3) *sn oc/ct*^{n} *sn*^{3} *sn*^{2} and *sn*^{1} *oc/cm ct*^{6} *sn*^{2}. If there were high negative interference one would expect wild type flies to be recovered from experiment (1) and in experiments (2) and (3) more wild types to be recovered from the two singed alleles than from the three singed allele heterozygotes. Results to date are: (1) 0 wild types/912,867 flies tested and (2) *sn*^{3} *oc/cm ct*^{6} *sn sn*—32/92,253 while *sn*^{3} *oc/cm ct*^{6} *sn*^{2}—37/152,451. The difference between these two results is

not significant ($P > 0.05$). Thus far no evidence for high negative interference is observed. In addition every one of the 69 wild type flies from experiment (2) was associated with recombination of the outside markers as predicted by the known order of the alleles, thus giving no suggestion of gene conversion or other unusual phenomena. No data are yet available for experiment (3) due to the unexpected sterility of one of the heterozygotes. (Supported by U. S. Public Health Service Grant C-3114[C–3].)

HILDRETH, P. E., Lawrence Radiation Laboratory and Department of Zoology, University of California, Berkeley: *Temperature shock and crossing over in fertilized eggs of Drosophila melanogaster.*—Inseminated eggs of *D. melanogaster* were collected over periods of five minutes and given a cold shock (—9 to +1°C) or a heat shock (35 or 40°C) for five to 15 minutes. The control eggs received no temperature shock. When shocked, the majority of the eggs were undergoing meiosis. The X chromosome from the female carried inversions, while that from the male did not, therefore detection of possible somatic crossing over in early cleavage cells would be infrequent.—In the controls three mosaic flies were observed among 17,901 individuals (0.166 per 1000); among the high-shocked eggs the ratio was 22 per 13,844 (1.59 per 1000); and among the low-shocked eggs 19 per 12,862 (1.47 per 1000). The mosaics ranged from females that had a small patch of apricot-colored tissue in an otherwise red eye to individuals that consisted of half male and half female tissues. These mosaics resulted from crossing over in an early developmental stage between the X chromosomes, which lead to different genotypes of sister nuclei. Frequently the crossing over resulted in the loss of a chromosome which would account for the male tissues present in an individual that began life as a female.—The frequency of triploids was about eight times greater in both the high and low-shocked eggs than in the controls indicating that either type of shock treatment will increase the frequency of meiotic segregation failures. (Research performed under the auspices of the U. S. Atomic Energy Commission.)

HOUSE, VERL L., Ohio State University, Columbus, Ohio: *Mutant effects in multiple heterozygotes of recessive venation mutants in Drosophila melanogaster.*—Apparent superadditive interactions, relative to L_4 interruption, characterizing multiple homozygotes of certain recessive mutants in Drosophila become additive on an assumed scale of vein-forming potentiality involving the operation of a threshold for mutant effect. The transformation of scale raises the question of the dominance relationships at the various loci involved relative to the new reference frame. In the absence of complete dominance a heterozygote would be buffered against a mutant phenotype to a lesser degree than the homozygote for the normal gene. In this case one would expect an augmentation of homozygous mutant effect at one locus by heterozygosity at a second locus. This type of result is found at both 18°C and 26°C for a variety of genotypic combinations. In addition, L_4 interruption characteristic of $H/+$ is greatly enhanced in the combination, *en*/+;$H/+$, and hemizygosity of the normal *ci* allele greatly augments the interruption characteristic of *en/en*. The lowered vein-forming potentiality characteristic of heterozygotes suggests the possibility of a transgression of the threshold for mutant effect in multiple heterozygotes. A search for venation effects in *en*/+; *ci*/+ was negative over the range of temperatures 14°C to 30°C, the combinations *cg*/+; *ci*/+ and *cg en*/+ +, however, showing 15.3% and 26.1% of L_4 thinning respectively for males at 18°C. The percentage of L_4 interruption or thinning is increased to 49.0% in the triple heterozygote, *cg en*/+ +; *ci*/+, and to 95.8% in the combination, *cg en*/+ +; *ci*+/—. In addition, the effects of heterozygosity at any two loci are cumulative on the transformed scale relative to the enhancement of L_4 interruption associated with homozygosity at a third locus. (Work supported by a grant from The National Science Foundation.)

HOWE, H. BRANCH, JR., Department of Bacteriology, University of Georgia, Athens, Georgia: *Biochemical mutants of Neuropsora tetrasperma.*—Unisexual strains of both mating types were isolated from the Dodge secondarily homothallic wild type strain 16387 kindly furnished by DR. L. S. OLIVE. Unisexuals were obtained by the method of SHEAR and DODGE (1927) in which infrequent, abnormally small ascospores (about 25 microns long) are selectively isolated under a

stereomicroscope from large numbers of ascospores randomly spread on dissection plates. Eighty-two of 119 isolates germinated, yielding 65 unisexuals, 35 of them *A* and 30 *a* as to mating type. The remaining 17 germinants selfed.—Sixty-four mutants for ten different biochemical requirements were obtained by selective plating following ultraviolet treatment and filtration-concentration of conidia of one of the *A* strains. Histidine mutants occurred most frequently, followed by pantothenic acid and methionine requirers. Heterokaryon tests indicated probable allelism within six of the ten requirement groups.—Considerable infertility has been encountered in attempts to cross the various mutants to the *a* strains. To date only *hist*, *pan*, *inos* and *ad* mutants have been fertile. Preliminary genetic analyses based upon frequencies of asci having homokaryotic ascospores indicate close centromere linkage for one of the *pan* mutants but rather loose centromere linkage for one of the *ad* mutants.

Hsu, K. S., Stanford University, Stanford, California: *The effect of the Dissociation-Activator system on mutation in maize.*—In maize, mutations can be induced by use of intranuclear controlling elements. Some elements appear to affect only a single locus while others affect several loci. McClintock (1950, 1951, 1953) has obtained evidence that the *Ds–Ac* system affects genic expression at a number of functionally dissimilar loci. The present experiment was designed to determine the number and kind of mutants which could arise through the operation of this system, and as a measure for the possibility of recovering unknown locus by the system, the design favored, by using homozygous waxy stocks, the detection of high-amylose mutants (Coe, 1957).—Plants carrying *Ds* and *Ac* were either crossed with waxy inbreds, with each other, or selfed. Observations were made in F_1 for dominant mutants and in F_2 for endosperm and seedling mutants. Most of the mutants recovered were of the stable recessive type which would not respond to *Ac*. The responses to *Ac* were not clearly established for one dominant morphological mutant and one endosperm mutant which was not involved in a high-amylose production. Reverse mutations were distinctly expressed on eight chlorophyll mutants which were found among about 17,500 gametes tested. These included one yellow, one yellow-green, one virescent, and five albino mutants. In each case, the presence of *Ac* was either readily recognizable, or proved by later tests. Three of the eight mutants each segregated 12 normal:3 mutable mutant:1 stable mutant seedlings which suggested *Ds* initiation, with *Ac* controlling mutability at an unlinked position. Two of the eight segregated only mutable mutant seedlings in the small populations tested. These could be interpreted as cases of autonomous mutability, or as *Ds*-initiated cases with the gene-*Ds* complex linked with *Ac*. The remaining three gave frequently an excess of the stable class over the mutable class. (Research performed at the University of Missouri.)

Huang, P. C., California Institute of Technology, Pasadena, California: *Functional complementation in carotenoid synthesis in vivo among several albino mutants in Neurospora crassa.*—Among ten albino mutants of *Neurospora crassa* obtained from Dr. D. D. Perkins, certain pairs appear to be functionally complementary in the synthesis of carotenoids *in vivo*. Two complementary strains, when inoculated on opposite sides of a Petri plate on Vogel's medium at 25°, produce normal pigmentation in the area of contact after 84 hours. Inoculations with a mixture of heavy conidium suspensions of these two strains result in a pigmented culture, in contrast to albinistic when only one is inoculated. In one combination analyzed (al^cA and al^aA), the pigments produced are qualitatively the same as those carotenoids normally present in the wild type strains. These carotenoids include, in the epiphasic fraction, spirilloxanthin, lycopene, neurosporene, zeta-like carotene, gamma carotene, beta carotene, phytofluene, and phytoene. Only the latter two polyenes are found to be present in the albino mutants. The identification of these carotenoids is based on their solubility, adsorptibility on the chromatographic column, eluatibility, and spectrophotometric characteristics. Carotenoids produced by other combinations are being analyzed. Heterokaryotic and genetic tests for allelism among these mutants and their functional and genetic relationships to the albino-1 (aurescent) and albino-2 mutants will be discussed.

IIZUKA, MUNEO, (Introduced by JULES JANICK), Kyoto University, Kyoto, Japan: ***Meiotic irregularities caused by inbreeding in Brassica and Raphanus.***—Although monogenomic cruciferous vegetables, such as *Brassica pekinensis* (AA, $\underline{x} = 10$), *B. rapa* (AA, $\underline{x} = 10$) and *Raphanus sativus* (RR, $\underline{x} = 9$) are usually self-incompatible, inbred lines can be obtained by means of bud pollination. In the process of inbreeding a number of abnormal characters generally appear. A study of inbreeding in Brassica and Raphanus also revealed abnormalities associated with meiosis. In the I_1 to I_{10} generations of selfing, a number of plants segregated which showed coenocyte formation and/or monocotyledon type cytokinesis. Coenocytes were formed in premeiotic mitoses as a result of nuclear division without cytokinesis. The frequency of coenocytes in pollen mother cells showed a seasonal variation being higher in spring than in summer. In some abnormal plants an intermediate type of cytokinesis and cell wall formation in the telophase of first meiotic division were observed, as in monocotyledonous plants. This abnormality varied considerably depending on both genotype and environment. Coenocyte formation and monocotyledon type cytokinesis were shown to be controlled by several independent recessive genes. Other meiotic irregularities as loosely paired bivalents, univalents, chromosome bridges, and localized nuclear plates were observed. These abnormalities appear to be an effect of altered genetic homoeostasis.

IVES, P. T., Amherst College, Amherst, Mass.: ***Further tests of mutation frequencies in successive sperm broods after 1kr of γ-rays.***—Virgin Oregon-R/hes males, irradiated at 48–60 hours after eclosion, were mated singly to six *vg se* females daily. F, males were tested for Y, 2 and 3 chromosome translocations. A marked peak in frequency (10–15%) occurred in days 5 and 6 sperm, with $< 1\%$ in days 7–12. Oregon-R/rucuca males irradiated at 0–12 hours showed a similar frequency pattern except that the peak (19%) appeared in day 7 sperm. That posteclosion age by itself did not cause this pattern change was shown by testing irradiated 0–12 hours, 48–60 hours and week old virgin Oregon-R/ruc-*th* (rucuca without *th*) males. Each age group showed a pattern like the 48–60 hours Oregon-R/hes. Simultaneous tests of 0–12 hours Oregon-R/rucuca and Oregon-R/ruc-*th* are in progress.—For tests of the T frequency in early *vs.* late emerging F_1 of irradiated males, flies from noncrowded cultures sired by day 5 sperm (24-hour laying period) were collected daily. The frequency was only half as high in F_1 males emerging in day one (roughly 20% of the total F_1 male hatch) compared to later days none of which differed from each other. Parallel X-linked mutation frequencies in semisibling F_1 females collected daily and tested by Basc showed no differences between days. F_1 samples are routinely taken in standard mutation rate tests here when more than 95% of the flies have emerged.

JANICK, JULES, and MUNEO IIZUKA, Purdue University, Lafayette, Ind.: ***Sex determination in a spinach line containing a heteromorphic sex chromosome.***—A single cross of *Spinacia oleracea,* derived from plant introduction accession 169671, has been shown to segregate for a morphologically distinct variant of chromosome 1. Chromosome 1 has been shown by trisomic analysis to contain the sex-determining factors *XY* in dioecious types. This chromosome, the longest of the complement, is normally heterobrachial, one arm being twice as long as the other. The nonstandard chromosome is isobrachial, apparently due to the addition of a segment to the short arm. The segment is presumed to be a translocation from another chromosome of the complement because all heteromorphic plants show quadrivalent pairing at metaphase I of meiosis in most cells.—The original line in which this isobrachial chromosome was observed, segregated into four equal classes in regard to sex and constitution of chromosome 1, *viz.* monoecious and heteromorphic, monoecious and homomorphic for the standard chromosome, pistillate and heteromorphic, and pistillate and homomorphic for the standard chromosome. Crosses and selfs involving heteromorphic plants indicate equal transmission of the two types of chromosome 1. No obvious morphological effects were associated with either 1 or 2 doses of the isobrachial chromosome. Genetic analysis of this line indicates that monoecism is determined by a single gene, X^m, which is assumed to be "allelic" to the *XY* pair. The phenotype of X^mX^m is monoecious with a preponderance of staminate flowers, X^mX is monoecious with a tendency for a preponderance of pistillate flowers, and *XX* is completely pistillate. The *X* gene was shown to be associated with

the isobracial chromosome. Although crossover types are believed to have occurred, recombinant gametes account for less than one percent of the total. (This study supported by the National Science Foundation G 14150.)

JUDD, B. H., University of Texas, Austin, Texas: *Structural and functional relationships between the zeste and white loci of Drosophila melanogaster.*—Asymmetrical exchange within the complex locus white results in products which are duplicated or deficient for a portion of the locus. Both products appear to be cytologically normal. Reciprocal exchange strands have been recovered from attached-X females heterozygous for apricot (w^a) and buff (w^{bf}) and appropriate linked markers. The duplication product ($w^{r,dup}$), which is "light-buff" in phenotype, acts as an allele of both white and zeste even though the zeste and white loci are separated by some 11 bands on the salivary gland chromosome map and by 0.5 units on the genetic map. The deficiency product ($w^{r,def}$), which is white in phenotype, acts as a white allele with a suppressor effect on zeste.—Exchange products from $y^z\ w^{r,dup}\ ec/+$ ♀ ♀ are (1) $y^z\ w^{bf}$; as yet, no apricot has been recovered. (2) $y^z\ w^{+,dup}$, (3) $w^{+,dup}\ ec$. Types (2) and (3) are wild type in eye color but both act as alleles of zeste. The $y^z\ w^{+,dup}$ product seems to exhibit a position effect with zeste, since $w^{+,dup}/z$ is more like wild type than is $z\ w^{+,dup}/+$.—The hypothesis is that the pseudoallelic white locus arose by intralocus duplication and that the zeste locus may have evolved by a similar process as a part of the same complex. The white and zeste regions have retained their synaptic homology since they are demonstrated to pair with each other is some cases (GREEN 1959). A functional relationship between the two loci has been shown by GANS (1953). (Supported by grant RG-6492 from the National Institutes of Health.)

KALTER, H., Children's Hospital Research Foundation, Cincinnati, Ohio: *On the possible origin of a strain difference between C3H and CBA mice.*—The C3H/J and CBA/J strains of inbred mice have been found to be greatly different in their teratogenic response to maternal treatment during pregnancy with cortisone, the former giving 63.2% young with cleft palate, the latter, 10.6%, although resorption frequencies were almost identical: 41.0% and 40.1%.—These two strains originated over 40 years ago, from offspring of a cross ♂ DBA × ♀ Bagg albino (STRONG, Cancer Research 2:531, 1942). The female came from a strain then being bred by pen matings, which by pedigreed sib matings later became the Strong A strain, from which the various A substrains descended. Two of these, A/J and A/He, and a substrain of the type to which the male belonged in cross mentioned above, DBA/1J, are all extremely susceptible to the cleft-palate inducing action of cortisone, generally giving 90–100% defective young. Yet the C3H/J and DBA/J strains, related to these others by common ancestors, are different from them and from each other.—There are several possible origins of this diversity: (1) The genetic heterogeneity of the original Bagg albino female, evidenced by her having borne F_1's that were black and brown agouti and nonagouti. (2) The selection the original offspring and their early descendants were subjected to (above citation). (3) Mutations arising during the 100 or more generations separating the present day strains. (4) Combinations of these possibilities. (This study was supported in part by grant RG-7107, N.I.H., U.S.P.H.S.)

KELTON, DIANE E., Zoology Department, University of Massachusetts, Amherst, Mass.: *Effect of the dilute-lethal gene (d^l) on sex ratios in mice.*—Examination of breeding data from mice heterozygous for d^l indicates that this allele may influence the sex ratio of offspring. Ratios were: D/D, 48.8% males; D/d^l, 54.5% males*; d^l/d^l, 61.4% males*.—Further study is needed to determine whether this is due to selective prenatal death, alteration of primary sex ratio such as studied by WEIR (Genetics 45: 1539, 1960), or selective fertilization as shown in t alleles by DUNN (Am. Naturalist 94:385, 1960) and BRADEN (J. Cellular Comp. Physiol. 56, Suppl. 1:17, 1960).—* Indicates statistically significant deviation. (Supported by U. S. Public Health Service Grant RG-5921.)

KELTON, DIANE E., Zoology Department, University of Massachusetts, Amherst, Mass.: *Myelin degeneration in the central nervous system of dilute-lethal mice.*—Brains and spinal cords of

dilute-lethal mice (d^l/d^l) were studied from four to 24 days of age using Pal-Weigert and Marchi methods to demonstrate formed and degenerated myelin.—Generally, myelination in d^l/d^l mice was simultaneous with that in controls (D/D and D/d). The cerebellum showed the earliest and most widespread degeneration; by 11 days the somato- and vestibulo-cerebellar systems were almost completely involved. Thereafter demyelination became increasingly severe. No degeneration was seen in olfactory lobes; in the cerebrum the putamen and tectospinal tract, and in the medulla the pyramidal tract and its decussation contained Marchi granules. The dorsal, lateral and ventral funiculi of the spinal cord anterior to the fourth cervical vertebra were demyelinated. Control mice exhibited no degeneration. (Supported by U. S. Public Health Service Grant RG-5921.)

KIDWELL, J. F., A. E. FREEMAN, L. H. HAVERLAND and G. M. H. ROLFES, Iowa State University, Ames, Iowa: *Genotype environment interaction in Tribolium.*—A knowledge of the existence and nature of genotype environment interaction is of importance in studying the genetics of natural and domestic populations. Experimental studies utilizing populations of laboratory animals provides a logical approach to these problems. This report describes such an experiment with the flour beetle, *Tribolium castaneum.* Ninety-three matings of one male and three females were made at random from a large panmictic population of Tribolium. The females were transferred to individual vials after four days. Six daughters were randomly selected, as pupae, from each female and individually mated to unrelated males. Three of the six were placed in an incubator (92°F, 45% relative humidity) and three in a cabinet at room conditions (72–88°F, 30%–35% relative humidity) and allowed to produce eggs during the 9, 10, 11 days of age. Progeny were counted as pupae and larvae 23 days later. In the analysis two measures of production were used. Total production was measured as the sum of the pupae and larvae and rate of development was measured as pupae. A conventional least squares analysis was employed. A large difference (40%–45% of the total variance) due to environments was observed. In the incubator the mean number of pupae and of pupae plus larva was 13.4 and 20.1 respectively, while the corresponding figures for room conditions were 3.3 and 9.2. Estimates of genotype environment interaction are provided by the Environment × dams in Sires and the Environment × Sires components of variance. Both were significant, but small for pupae, accounting for 5.75 and 2.88 percent of the total variance. Only the Environment × dams in Sires Component was significant for pupae plus larvae, and it accounted for 3.80 percent of the total variance. Two estimates of heritability are available, one from the sire and one from the Dams in Sires Component of variance. These estimates were 0.37 and 0.20 for pupae and 0.28 and 0.21 for pupae plus larvae. (This work has received assistance from Contract AT[11-1]107 from the Atomic Energy Commission. Journal Paper No. J-4111 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1187.)

KIMBALL, R. F., and STELLA W. PERDUE, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Further studies on the refractory period for mutation induction in Paramecium aurelia.*—The postduplication interphase (G2) and early prophase micronucleus of *Paramecium aurelia* has been shown to be extremely refractory to the induction of recessive lethal and slow-growth mutations by radiation. Studies on the intracellular symbiont, kappa, suggest that this refractoriness is a specific property of the micronucleus and not the consequence of an over-all refractoriness of the whole cell and its contents. Mutation induction by the thymidine analogue, 5-bromodeoxyuridine, shows a very different pattern; and this, together with other considerations, makes probable the interpretation of the refractory period as a time of very efficient repair of localized radiation-induced lesions in the chromosomes. The small amount of lethal mutation induced during G2 shows that isochromatid aberrations are rare or missing. This is in agreement with other evidence that chromosome aberrations of all kinds are rare or absent in this material no matter when the paramecia are irradiated.

KITZMILLER, J. B., and W. J. KEPPLER, University of Illinois, Urbana, Ill.: *Salivary gland chromosome maps in Culex p. pipiens.*—Salivary gland chromosome maps have been prepared

for the mosquito *Culex p. pipiens*. The strain used was originally collected in the field in Champaign, Illinois. The salivary chromosome complement consists of three pairs of synapsed, polytene, banded chromosomes. Each pair is usually closely synapsed but asynaptic regions have been observed in all three pairs. The bands vary in intensity of staining and occur in definite patterns. All chromosome ends can be recognized by shape and banding patterns. Each pair consists of an indistinctly banded central portion, probably the centromere region, and two arms of approximately equal length. Average measurements in microns for each chromosome are: I, left arm, 150; centromere, 20; right arm, 165: II, left arm, 170; centromere, 20; right arm, 225: III, left arm, 200; centromere, 30; right arm, 225. The shorter arm has in each case been arbitrarily called the left arm. Following the Drosophila convention, each chromosome has been divided into numbered regions, with subdivisions of each region designated by letters. Chromosome I contains regions 1–22; chromosome II, 23–50; and chromosome III, 51–80. Each arm contains several prominent banding sequences which provide distinct identification. Each chromosome contains a central, "double bulb," with indistinct bands. This region has tentatively been identified as the centromere region, and good preparations show bands suggestive of a reversed repeat pattern. Meiotic chromosomes (testis, metaphase I) measure 6.2, 7.7 and 8.5 microns, with median centromeres and arms of approximately equal length. There are no recognizable heterochromosomes. The salivary maps herein reported have been designated as "standard" maps and are being used for comparison of the patterns in *Culex p. fatigans* and in several X-ray mutants. (Supported by grant E-3486 from the U.S.P.H.S.)

KODANI, M., Rutgers University, New Brunswick, N. J.: *Alteration of blood group types in human amniotic cells cultured in heterologous blood group substances.*—Human amniotic cells cultured *in vitro* are shown by the COOMBS' mixed agglutination technique to contain blood group substances corresponding to their ABO blood groups. Amniotic cells cultured in the presence of heterologous blood group substances have been found by the same technique to acquire the capacity to fix blood group antibodies against the heterologous antigens to which the cells are exposed. Various experimental tests indicate that the capacity acquired by the cells is due to the coating of the cells with the heterologous blood group substances. Whether this phenomenon is a nonspecific one or involves a specific binding of the heterologous antigens and certain structures of the cells is not yet clear. Experiments are now underway to investigate this problem.

KOSIN, I. L., MASARU KATO, and S. H. WEISBROTH, Washington State University, Pullman: *Absence of detectable hereditary changes in White Leghorn chickens after three generations of intra- and interspecific blood injections.*—This investigation had its genesis in PROFESSOR H. F. KUSHNER's (USSR Institute of Genetics) account, at 10th International Congress of Genetics, of his successful inductions of hereditary changes in chickens by heterologous blood injections.—We also used his procedure of massive blood infusion (intraperitoneally) either from Broad Breasted Bronze turkeys (modified black plumage) or from New Hampshire chickens (reddish-brown) into separate lots of White Leghorn chickens (dominant white). The base (P_1) generation came from pure-breeding White Leghorns maintained at WSU since 1946. At hatching, P_1 chicks were randomly divided into control (noninjected) and experimental (injected) lots, to produce corresponding treatment lines. In F_1 and F_2, all definitive matings were made on within-line basis. In P_1, F_1, and F_2 the experimental chicks began receiving whole blood injections when four days old, which continued on twice-a-week basis for 20 weeks. Each experimental bird by then received 150 milliliters of heterologous blood. Observations were kept on plumage color, comb shape, body weight and shell color. Furthermore, F_1 and F_2 were subjected to comparative immunological (precipitation, agglutination and OUCHTERLONY's procedure), electrophoretic and chromatographic analyses, based on blood and muscle tissues. Again, the aim was to check for the possible presence of any hereditary shifts in the experimental birds. The total number of birds involved in each generation, by treatments (experimentals and controls, in that order) was: P_1, 50–50; F_1, 490–104 (plus 263 reciprocal crosses); F_2, 1180–937.—So far, the results have been negative on all counts pertaining to KUSHNER's thesis.

KRIVSHENKO, JAKOV, Department of Biology, University of Rochester, Rochester, N. Y.: *The chromosomal polymorphism of Drosophila busckii from natural populations.*—As a result of the cytological analyses of the progeny of the *D. busckii* flies from the populations of Princeton, N. J. and Rochester, N. Y., as well as the analyses of the progeny of separate lines from several geographically remote populations, six different chromosomal aberrations were found. One of them (P-X) represents a simple inversion of the middle part of the X chromosome; its frequency is 5% when computed from the number of analyzed X chromosomes. Another chromosomal aberration represents a complex consisting of five inversions located in IIIL. The remaining four aberrations represent variations of complex inversions of the second chromosome. One of these complexes (P-A) consists of nine inversions; four are located in the left arm and five in the right arm. The frequency of this complex is 3%. The second complex (P-A + R_1) consists of the inversions of the preceding complex, and one new inversion located in the right arm. The frequency of this complex is 0.28%. The third complex (P-A + R_2 + R_1) includes all inversions already mentioned and one additional inversion which is also located in the right arm. Thus, this complex consists of 11 inversions. Its frequency in the population is 0.55%. Finally, the fourth complex differs from the preceding one by the absence of one inversion in the left arm, which is evidently due to crossing over. This complex was found in one of the lines from Bogota, Colombia. All inversions in these aberrant complexes are small. However, the particular characteristic of the complex inversions of the second autosome is the presence of adjacent inversions sharing the same breaks; three of them are located in the left arm and four in the right arm.

The fact that identical aberrations were found in geographically remote populations (Princeton, N. J., Rochester, N. Y., Texas, and Colombia) in different years of investigation (1952–1960) indicates that the occurrence of these forms is not accidental and that they represent permanent elements in these populations. Their wide distribution in North and South America is presumably due to their peculiar genetic characteristics. (This investigation was supported by a research grant RG-3934[C6] and RG-3934[C7] from the National Institutes of Health, Public Health Service, to the University of Rochester.)

LACHANCE, LEO E., Entomology Research Division, U.S.D.A., Kerrville, Tex.: *Radiosensitivity of the various stages of oogenesis in Callitroga hominivorax.*—The female reproductive system of this insect affords an ideal opportunity to study a large number of germ cells all of which are in the same stage of oogenesis. The female usually will not oviposit until seven days old. During this time approximately 250 germ cells are undergoing growth and maturation, each in a separate ovariole.—Virgin females ranging in age from a few hours to seven days old were irradiated with 2650r of gamma radiation, then mated to untreated males and egged when they were eight days old. At this time the females deposited approximately 250 eggs. Failure of the eggs to hatch was attributed to dominant lethal changes induced in the oocyte. Radiosensitivity of the developing oocytes varied considerably. Irradiation of the females less than four hours old resulted in complete sterility. Mature oocytes were not formed. Observed hatchability for the other age groups was females 18–24 hours old, 30%; more than 24 hours old, 60%; 2 days old, 64%; 3 days, 76%; 4 days, 30%; 5 days, 14%; 6 days, 21%; and 7 days, 17%.—Cytological investigations of the ovarioles indicated that the high radiosensitivity during the first day corresponds to changes taking place in nurse cell nuclei during the first 24 hours of adult ovarian growth. Trophocyte units are completely differentiated after 24 hours and this confers a degree of radioresistance to the oocyte-trophocyte group. After the third day the oocyte is almost fully mature and nurse cells have disappeared. Increased radiosensitivity of the oocyte on the fourth day, and after, is then due to changes taking place within the oocyte nucleus.

LANDMAN, OTTO E., and HERBERT S. GINOZA, U. S. Army Chemical Corps, Fort Detrick, Frederick, Md.: *Cytoplasmic and nuclear control of cell division and cell wall formation in bacteria.*—When a population of *Salmonella paratyphi* is treated with 40–200 units/ml of penicillin, up to 50% of the cells survive to give rise to L-colonies; the remainder are killed. The Salmonella L-colonies are stable, i.e., when penicillin is withdrawn they do not return to the

bacillary state, except for exceptional reversions which occur at a frequency of less than $1/10^8$. The L-colonies consist of soft, spherical bodies which grow only in soft serum agar but are unable to divide in liquid media. Clearly, both ability to form the rigid cell wall and ability to divide are heritably impaired in the L-form. Rigid wall formation, and to a lesser extent cell division, are also inhibited with 10 units of penicillin/ml, but spheroplasts or L-forms formed at this penicillin concentration can still divide in liquid media and promptly revert to the bacillary state upon withdrawal of the antibiotic.—Resistance to rigid-wall-formation-inhibition by penicillin can mutate independently from resistance to division-inhibition by penicillin. By contrast, single mutations also can increase the resistance to both division-inhibition and wall-inhibition.—The pleiotropic effects of penicillin can be explained if it is assumed that a single bacillary organelle is responsible for both septation and rigid wall formation. A plausible candidate organelle is the bacillary membrane. The preceding evidence indicates that it exhibits both autonomous and nucleus-dependent genetic properties.

LANGHAM, DERALD G., Yale University, New Haven, Conn.: *The High-Low concept of biological systems.*—The latitude of expression of a given character in a natural population may be said to have an upper limit (HIGH) and a lower limit (LOW). Evidence that genes (*H*) with a strong potential to exceed the High limit and others (*L*) with a strong potential to exceed the Low limit are resident in the germplasm has been obtained in sesame (*Sesamum indicum* L.). Their failure to express themselves indicates they are buffered in the biological systems. Experiments have demonstrated that the triggering mechanism for their release is at the opposite end of the curve. By crossing High × Low representatives of the population and growing large F_2's, the *H* genes segregate free of their specific buffers (as do the *L* genes) and strong transgressive segregation results. If these new extremes are represented by *H′* (*H* minus its buffers) and *L′* (*L* minus its buffers), crosses of *H′* × *H′* and *L′* × *L′* are indicated to accumulate genes affecting the same character or complementary characters. By crossing *H′* with the Low zone and *L′* with the High zone the resulting F_2's yield a new High (*H″*) and a new Low (*L″*). These results are interpreted as meaning that the specific buffers for *L* genes become *enhancers* when in combination with *H′* genes, and that the buffers for *H* become enhancers for *L′*. Crossing *H″* × *H″* and *L″* × *L″* gives further advance in the respective directions.

LEWIS, H. W., and H. S. LEWIS, Massachusetts Institute of Technology, Cambridge, Mass.: *Factors on chromosome II and III involved in the control and regulation of dopa oxidase activity in Drosophila melanogaster.*—The three lines with distinct dopa oxidase activity, *lo-2*, *lo-3* and *hi-3* (LEWIS and LEWIS Proc. Natl. Acad. Sci. U.S. 47:1, 1961) were crossed with a *B*; *Cy/Pm*; *Sb/Ubx* balanced marker stock and testcross and F_2 classes were studied to determine the roles of chromosomes I, II, and III in the expression of dopa oxidase activity. Testcrosses involving *lo-2* (homozygous alpha) indicate that (a) chromosome I is not associated with a detectable modification of enzymatic activity, (b) the three chromosome II combinations have distinctly different levels of activity expressed as dopa oxidase units (*Pm/α*, 510 ± 120; *Cy/α*, 200 ± 50; *α/α*, 60 ± 20) and (c) the *Sb* marked chromosome III modifies the activity in the *Pm/α* class (*Pm/α*;*Sb/+*, 370 ± 50 : *Pm/α*;+/+, 580 ± 80). Comparison of the classes of the testcross involving the *lo-3* line suggests minor differences between the overlapping activities in the *Pm/+*, *Cy/+* and +/+ classes and within these classes, minor differences between *Sb/+* and +/+. The pattern is the same in the comparison of the classes of the testcross with the *hi-3* line, but there is no overlapping between the two lines (*lo-3* testcross, 200 ± 50; *hi-3* testcross, 620 ± 130). Significant differences have been found in the F_2 classes grouped according to second chromosome markers in crosses involving the *lo-3* line (*Cy/Pm*, 610 ± 80; +/+, 190 ± 40) while the corresponding classes in crosses with the *hi-3* line are not distinguishable from each other. The F_2 and testcross data indicate that a major difference between the *lo-3* and *hi-3* lines is in chromosome II and that the presence of one second chromosome from the *lo-3* line results in low dopa oxidase activity. The marked modifying effect of the *Sb* chromosome in testcrosses involving the *lo-2* line and the indication of minor differences between *Sb* and non-*Sb*

classes in testcross populations of *lo-3* and *hi-3* suggest that the latter two lines, derived by selection and inbreeding from a common stock may differ with respect to chromosome III modifiers in addition to their second chromosome differences.

LÖBBECKE, ERNST-ARNOLD, and R. C. VON BORSTEL, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Differential sensitivity of Habrobracon oocytes in the first meiotic prophase and metaphase to chemical mutagens.*—It has been demonstrated that Habrobracon oocytes in the first meiotic metaphase are much more sensitive to nitrogen mustard than oocytes in the first meiotic prophase when dominant lethality is criterion. A large sensitivity difference also obtains when ethyl methane sulfonate is used. This is in accord with X-irradiation data on Habrobracon. After treatment with nitrogen mustard, oocytes in the first meiotic metaphase are also more sensitive than oocytes in first meiotic prophase with respect to induction of recessive lethal mutations. In preliminary experiments, at a dose where hatchability of unfertilized eggs was 54% for metaphase I and 89% for prophase I, the embryo recessive lethal frequency was 6% for metaphase I and less than one for prophase I. The experiments are being repeated and extended to compare the results with those from X-irradiation experiments.

LUCE, WILBUR M., University of Illinois, Urbana, Illinois: *Effect of lactamide and other chemicals upon the facet number in the mutant forked infrabar in Drosophila melanogaster.*—Previous work chiefly by DR. S. KAJI and DR. M. OGAKI has shown that certain chemicals when fed to larvae at definite times markedly increase the facet number of mutant Bar in *Drosophila melanogaster*. This is a report of similar work feeding chemicals to larvae of forked infrabar (fB^a) flies. Infrabar (B^i) is an allele of Bar with a facet number considerably higher than that of Bar and much nearer wild type in expression. Forked (f) is a bristle mutant used as an aid in detecting contamination. Larvae were transferred to the experimental vials containing food mixed with the proper amount of chemical when they were 67–73 hours old reckoning from the time of deposition of the eggs. The temperature at which the larvae were raised was 25± 0.2°C. Lactamide (2.8%) was especially effective in increasing facet number. With this treatment females had a mean facet number of 573±1.0. Forked wild-type flies at 25°C. had means of 731±2.3 for females and 689±3.6 for males. Other highly effective chemicals in their action on infrabar were ammonium lactate (13%) and magnesium acetate (6%). Other chemicals tried with less but significant effects were casein (3%), urea (4.5%) and thymine (1%). (The experimental part of this work was done in the Division of Biology at the California Institute of Technology, Pasadena, California.)

LUND, D. E., University of Nebraska, Lincoln, Nebr.: *Induction of CO_2 sensitivity by interspecific injections in the D. obscura group.*—The cytoplasmically determined CO_2 sensitivity of *D. affinis* (Nebraska) has been transferred by injection to resistant strains of *D. athabasca* (Wyoming-Quebec hybrid strain), *D. azteca* (Mexico), *D. miranda* (Idaho), and *D. pseudoobscura* (Mexico and British Columbia). Inheritance of such induced CO_2 sensitivity has been demonstrated in *D. miranda* and *D. pseudoobscura*. In *D. miranda* this CO_2 sensitivity has persisted in some individuals for more than six months, while a 100% CO_2 sensitive strain of *D. pseudoobscura* (Mexico) has been established and maintained for more than two years. (Supported in part by a U. S. Steel Fellowship.)

MAGNI, G. E. and R. C. VON BORSTEL, Istituto di Genetica, Universita' di Pavia, Pavia, Italy, and Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Spontaneous mutation rates in vegetative and sporulating yeast.*—The spontaneous back mutation rate per division has been determined in a diploid strain of *S. cerevisiae* homozygous for the methionine-requiring mutant met_{1-1}. The rates have been estimated during vegetative reproduction (mitosis) and sporulation (meiosis). The mutation rate per division ranged in different experiments from 3.4 to 4.8×10^{-8} while in parallel experiments during meiosis the rate was from 14 to 28×10^{-8}. The difference was statistically significant in all the experiments.—It was shown that the

selected prototrophs from the tests of the diploid cells and haploid spores were true back mutations at the specific locus and were not attributable to dominant or recessive suppressors.—The average generation time during vegetative growth (1 hr 12 min) can be increased by either glycerol (2 hr 36 min) or trycomycin (9 hr 24 min). The mutation rate per division remains unchanged under either circumstance, therefore the high frequency of spontaneous mutations occurring during sporulation cannot be attributed to the observed longer duration of time between divisions.

MAGUIRE, MARJORIE P., University of Texas, Austin, Texas: *Cytological evidence for premeiotic crossing over in maize.*—Rare events of probable premeiotic exchange of partially homologous segments have been observed in six of fifty-one of the 21 chromosome maize plants (grown simultaneously) which carried reciprocal Zea-Tripsacum interchange chromosomes. The exchanges are presumed to have occurred within a few cell generations preceding meiosis, since in each of the six plants only one or two (of many) microsporocytes showed the exchange. Exchanges were evidenced by the appearance of a terminal knob in a new position at pachytene. In the exchange sporocytes of five plants the knob was absent from its usual location on a Tripsacum segment but appeared instead on a partially homologous corn segment. In one microsporocyte of the sixth plant the knob appeared on both Tripsacum and corn segments suggesting that exchange occurred at a four strand stage and was followed by distribution of both knob bearing chromatids (one exchange, one parental) to the same pole at anaphase.—Since exchanges involved corresponding segments of partially homologous chromosomes, a crossover mechanism may have been involved. Due to preferential pairing of corn segments at pachytene the frequency of meiotic synapsis of Zea and Tripsacum segments was of the same order of magnitude as the peculiar exchange events between them. Cytological tests for premeiotic crossing over are available only in sporocytes where synaptic configurations involve partly paired, distinguishable chromosomes as in the material described here.—Alternative interpretations include transfer of the knob alone by some unknown mechanism, and separation and resynapsis of chromosomes following meiotic crossing over.

MARTIN, A., JR., VA Hospital, Leech Farm Rd., Pittsburgh 6, Pa.: *Serological evidence for a species genotype in the genus Vibrio.*—During the course of investigating the role of bacteria in the etiology of multiple sclerosis, a species of Vibrio was isolated from filtered distilled water used in the preparation of culture media, that has proven to be morphologically, biochemically, and serologically similar to the bacterium, *Spirochaeta myelophthora*, reportedly isolated from the cerebrospinal fluid of individuals diagnosed as multiple sclerotic. Complement fixation tests employing this latter organism as antigen, and an antigen prepared from the free-living waterborne saprophytic Vibrio, gave almost identical results with sera from multiple sclerotics (12+, 10—), other neurologics (10+, 3—), schizophrenics (6+, 11—), and nonhospitalized individuals (24+, 20—). To test the hypothesis that the Vibrio in question has world-wide distribution, complement fixation tests have been performed on sera from nonhospitalized individuals in widely separated geographic areas; Pittsburgh, and Philadelphia, Pa., London, and Brighton, England; with parallel results. The British tests gave 44 reactive, 84 negative, and the American tests gave 60 reactive, 35 negative. It would seem from these data that the Vibrio employed as antigen is as prevalent in England as in the United States, and is certainly indicative of a common genotype for this species. These data also support the hypothesis that the Vibrio in question is in no way involved in the etiology of multiple sclerosis, but is merely one of the many contaminants that may be encountered in bacteriological procedures. (This investigation was supported in part by Grant No. 261 from the National Multiple Sclerosis Society.)

MATHAN, DAVID S., University of California, Berkeley, Calif.: *Partial reversal of the effect of the lanceolate gene in tomato by the addition of tyrosine.*—Lanceolate, a spontaneous mutant in the tomato, has simple, lanceolate rather than the normal pinnately compound leaves. When lanceolate plants are selfed, the progeny segregates in a ratio of 1 normal:2 lanceolate (hetero-

zygous):1 homozygous lanceolate. Plants homozygous for the lanceolate gene appear in three forms: reduced, modified and narrow. Reduced is devoid of cotyledons and plumule and grows into a stub of 5 cm long and 0.2 cm in diameter. Modified has a flattened, cotyledon-like tip but fails to grow appreciably. Narrow has one or two cotyledons as well as plumule and grows into a plant comparable to lanceolate in size, which exhibits extreme lanceolate features. The leaves are simple and much smaller than in the lanceolate; in addition, instead of the pedicels terminating in flowers, they grow into tendril-like structures. The relative frequency of each of the homozygous phenotypes seems to depend on the age of the seeds. Fresh seeds gave more narrow plants than year-old seeds. However the predominant form in all cases is the reduced. Anatomical studies indicate that the meristematic tissues of shoot apices and leaves have progressively fewer and larger cells in the sequence normal, lanceolate and homozygous lanceolate.—In an attempt to induce growth in the reduced, embryos were cultured in sterile White's medium to which various growth substances were added. After it was found that casein hydrolysate converted some reduced embryos to narrow, single amino acids were added separately and it appeared that tyrosine alone gave the full effect of mixtures of amino acids. Tyrosine induced the formation of a cotyledon-like structure and a bud, which developed into a narrow seedling. When transplanted to soil, such seedlings continued to grow to give narrow plants without any further addition of tyrosine. Furthermore, when tyrosine was sprayed on the foliage of both naturally occurring and induced narrow plants, the leaves increased in size and approached those of lanceolate; in addition flowers appeared in place of the tendril-like structures. When leaves of lanceolate were sprayed with tyrosine they approached the normal both in size and increased number of leaflets along the midrib.—A quantitative bioassay of *Leuconostoc mesenteroides* of normal lanceolate and narrow shoot apices was undertaken. Preliminary results indicate that the soluble tyrosine content is highest in the normal, intermediate in the lanceolate and lowest in the narrow. (Supported by the Cancer Research Coordinating Committee of the University of California.)

McDonald, D. J., Department of Biology, Dickinson College, Carlisle, Pa.: *The effect of neutrons and X-rays on the fertility of Tribolium confusum.*—The effect of 180 kv X-rays and fast neutrons with an average energy of about 4 Mev, on the fertility of males of the flour beetle *Tribolium confusum* was investigated by mating each irradiated male with ten successive females for two-day periods and determining, in the X-ray experiments, the percent of viable eggs and in the neutron experiments, the number of offspring produced by the females over several days. 29.6% of the eggs produced by 120 females mated with ten males receiving 2900r of X-rays, developed into adults, compared to 39.9% for ten males receiving 1450r and 82.2% for ten unirradiated control males. Viability of eggs produced by females mated with X-rayed males 11 to 12 days after irradiation reached minimal values of 10.5% and 30.2% in the higher and lower dose experiments respectively, probably reflecting differences in the sensitivities of cells in various stages of spermatogenesis. Hatchability in the 2900r experiments tested 55 days following irradiation of the males, had attained control levels, indicating the absence of any permanent damage.—In the preliminary experiment with neutrons the mean number of offspring produced by the females mated with irradiated males was 59.3 compared to 77.0 for the controls. No significant difference in the fertility of the males during the 12 days following irradiation was noted. [Research supported by NSF Grant G–8888 and USPHS Grant RG–5836 (R1).]

McGregor, John F., and Howard B. Newcombe, Biology Branch, Atomic Energy of Canada Limited, Chalk River, Ontario: *Maze-learning ability in rat populations after a number of generations of gonadal irradiation.*—Maze-learning ability has been studied since August 1956 in offspring of irradiated and unirradiated rat populations. Three populations received doses of 600, 800, and 1000r (of 2 Mev X-rays) to the male gonads immediately prior to mating in each generation; the populations (and their three controls) consisted at the outset of 30, 30, and 60 mating couples, respectively. Data on learning ability are now available involving tests on a

total of 464 animals, extending over six weeks for each animal. Combined data on six generations of offspring from the 600r population (and its control) showed no significant effect of the irradiation. Combined data on four generations of offspring from the 800r population showed a reduced learning ability as compared with controls (13 percent reduction in the normally distributed score; $P = .06$); this effect was confined to males (22 percent reduction; $P = .02$). Combined data on two generations of offspring from the 1000r population also showed a decline in learning ability (13 percent reduction; $P = .04$); this was observed in both sexes (17 and 15 percent reduction in males and in females; $P = .09$ and .14). The interpretation is complicated by a reduction in litter size in the irradiated (but not in the control) populations, and by the possibility of genetic drift in both the control and the irradiated groups.

MENZEL, MARGARET Y., Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Department of Biological Sciences, Florida State University, Tallahassee, Florida: *Pachytene chromosomes of the intergeneric hybrid Lycopersicon esculentum × Solanum lycopersicoides.*—Chromosome pairing in the sterile F_1 is variable and incomplete; in the fertile amphidiploid most of the chromosomes pair as bivalents (RICK 1951, Proc. Natl. Acad. Sci. U. S. 37: 741–744). The pachytene chromosomes of the hybrid were compared with those of the parents to determine whether the chromosome differentiation assumed to underlie chiasma failure in the F_1 and preferential pairing in the amphidiploid is reflected in visible differences or synaptic failure at meiotic prophase. Pairing in the hybrid shows that the chromosomes of the parents are alike in 43 of the 47 identifiable regions into which the pachytene complement may be divided. The similar regions include all of the 23 distal, achromatic segments in which chiasmata are formed. In these regions synapsis is as successful in the hybrids as in the parents. Four of the 24 proximal, chromatic regions differ in length and hence show a "deficiency" configuration in the hybrid. According to the system of BARTON (1950, Am. J. Botany 37: 639–643) these are: the short (nucleolar) arm of chromosome 2, the proximal segments of the long arms of chromosomes 4 and 9, and the proximal segment of the short arm of chromosome 10. The inequalities in the chromatic regions probably account for the three or four unequal bivalents seen at metaphase I in the hybrid.

MERICLE, L. W., and R. P. MERICLE, Brookhaven National Laboratory, Upton, L.I., N.Y.: *Use of proembryos instead of dry seeds for the induction of mutations in cereal crops.*—When using ionizing radiation to increase the genetic pool available for selection, it is always advantageous to secure as large an isomutant test population as possible. In self-fertilizing cereal crops such as barley, where pollen irradiation is impractical, even though it theoretically might be the method of choice, dry seed irradiation has become the common procedure. By far the principal drawback to this latter method is the small size of the mutant-carrying sector induced and consequently, the small numbers of isomutant plants produced which often necessitates delay of testing and selection until the X-3 or later generations. While investigating the practicality of an alternative method which utilizes irradiation of proembryos developing within the ovaries of young spikes, we have recovered mutant-carrying sectors 2–20 times larger (involving 2–5, or even more, entire heads instead of one or only a part of one head) with a frequency and a fertility equal to or greater than that usually found following dry seed irradiation. As a consequence, the size of the isomutant test population is also increased at least 5–25 fold. Despite the more involved handling during radiation treatment, the use of proembryos instead of dry seeds in artificially induced mutation breeding programs could in many instances, therefore, result in an over-all saving of time and space since the selection of less drastic mutations (requiring groups of isomutant plants for detection) or the screening for resistance to several different pathogens, can be inaugurated at least one generation sooner.—(Research carried out at Michigan State University and at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

MEYER, HELEN U., and H. J. MULLER, Indiana University, Bloomington, Ind.; *Similarity of X-ray-induced mutation rate in gonia of Drosophila females and males.*—Early gonia are

agreed to have the lowest X-ray-induced mutation rate of all germ cell stages of adult *Drosophila melanogaster*. However, reports have disagreed concerning whether their rates are alike in both sexes. Our present experiments have investigated the frequency of second chromosome recessive lethals induced in early gonia of both sexes X-rayed simultaneously with 4000r (100 kvp, 240r/min). Lethals induced in coisogenized chromosomes carrying *fes-ms-b-cn-sp*, of flies heterozygous for Curly-Oster, were detected by "criss-cross sterility" tests improved since MULLER's Drosophila Inform. Serv. description. Individually identified young females and males, breeding for three days just before irradiation, produced control progeny denoted "YFC" and "YMC," respectively, and, *after* a 10-day or 15-day postirradiation period of active breeding, produced experimental progeny denoted "10FX," "15FX" and "15MX," respectively. As another control, "15MC," progeny were taken from other males, unirradiated, after breeding them as long as those irradiated. Determination of induced lethal rate was made unusually accurate by allelism tests of lethals derived from the same parent before and after irradiation, some spontaneous mutations being thereby excluded from the "rectified" experimental rate. Allelism tests also disclosed "clustering" within experimental or control sibships, thus permitting more accurate standard error determinations (MULLER 1952, GSA Records). Harmonically weighted mean frequencies (MULLER 1941, Am. Naturalist) of six such experiments gave, for "unrectified" controls: YFC (3159 chromosomes tested) $.61 \pm .16\%$; YMC (2718) $.29 \pm .11\%$; 15MC (2282) $.17 \pm .08\%$. For "rectified" experimentals: 10FX (1962) $6.3 \pm .7\%$; 15FX (1441) $6.5 \pm .6\%$; 15MX (2029) $7.4 \pm 1.2\%$. These results, which for oogonia concur with our previous X chromosome results, show no significant sex difference. (Supported by U. S. Public Health Service N.I.H. RG-5286-C2,3.)

MEYER, JAMES R., and VESTA G. MEYER, Delta Branch Experiment Station and Agricultural Research Service, U. S. Department of Agriculture, Stoneville, Mississippi: *Cytoplasmic male sterility in cotton.*—Male sterility has been found in derivatives of cotton species hybrids which combine the cytoplasm of one species with the genes and chromosomes of another species. When the cytoplasm of the wild, lintless African species, *Gossypium anomalum* Warsaw and Peyr. ($2n = 26$), was combined with genes and chromosomes of an Asiatic cotton (*G. arboreum* L.: $2n = 26$), the anthers were usually modified into petal-like structures. These male-sterile flowers produced fully-developed bolls after hand pollination. A double haploid genome of Upland cotton (*G. hirsutum* L.: $2n = 52$) was transferred into cytoplasms of the diploid species, *G. anomalum* and *G. arboreum*, by backcrossing. The aborted anther type of male sterility was frequently found on vigorous, female-fertile plants derived from these backcrosses. After three and four backcrosses, some populations were completely male-sterile and others had a high incidence of male-sterile plants. Both the petaloid and aborted anther types of male sterility were probably due to cytoplasmic-genetic interaction (cytoplasmic male sterility).

MILLER, W. J., University of California, Davis, Calif.: *Evidence for two new systems of blood groups in cattle.*—From studies of three new blood factors N, R′ and S′ discovered in this laboratory, it has been observed that they segregate independently of the blood factors in each of the genetic systems named A, B, C, F-V, J, L, M, S and Z. Blood factors R′ and S′ segregate as genetic alternatives and have been observed in only three phenotypes, R′, R′S′ and S′. It is concluded, therefore, that R′ and S′ belong to a new genetic system designated R′-S′ which presently involves but two alleles.—Blood factor N segregates as an alternative to no-N and independently of R′ and S′. Consequently, another genetic system, designated N, is postulated, thereby bringing the total number of bovine blood-group systems to 11.

MITTLER, SIDNEY, Northern Illinois University, DeKalb, Ill.: *Development of resistant strains of tumor S 180 to chemotherapy.*—The sudden development of resistance of tumors to antitumor drugs is a constant problem in a chemotherapy program. Tumor S 180 which was maintained in random-bred albino Swiss mice was tested for development of resistance to the antitumor drugs, mustargen, actidione, and *n*-methylformamide. The S 180 tumor was implanted weekly

into three groups of 21 mice, 11 of one group were treated by intraperitoneal injections for six days with 200 mg/kg/day of *n*-methylformamide, another group with 1 mg/kg/ day of *n*-mustargen and the third group with 32 mg/kg/ day of actidione. The largest tumor was selected from each of the treated group to perpetuate the resistant strain and in turn was implanted again into a group of 21 mice. The treatment was repeated with the antitumor drug upon 11 of the mice. The response of the tumors to the treatment was measured by the ratio (T/C) of the total weight of tumors from treated mice to the weight of the tumors of control mice. The tumor strain treated with actidione developed resistance after five weeks of treatment. The initial T/C ratio of .23 increased to .69 and to .88 by the 12th week of treatment. This resistance was maintained without relapse after 19 weeks of transfer. The resistance to mustargen developed after the fourth week of successive treatment (from a T/C = .27 to .72) and the induced resistance in this tumor strain was stable after 12 weeks. Resistance to *n*-methylformamide was readily produced (T/C = .81) by the fifth successive week of treatment, however this resistant strain although challenged daily with *n*-methylformamide lost its resistance in the 11th week (T/C = .24), regained it during the 18th week (T/C = .75) and again became sensitive to the drug (T/C = .27) during the 21st week. The resistance to *n*-methylformamide developed again in the 23rd week and was still present by the 33rd transplantation period. Each of the resistant strains still retained their sensitivity to the other two drugs.

Monesi, Valerio, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn., U.S.A., and Biology Division of the National Committee for Nuclear Energy, Research Group for Embryology, Frascati (Roma), Italy: *Relation of X-ray sensitivity to mitotic cycle in spermatogonia of the mouse.*—The present study was undertaken in an attempt to elucidate the radiation response of spermatogonia of the mouse. F_1 hybrid male mice (C3H ♂ × 101 ♀) were injected intraperitoneally one hr prior to irradiation with H^3-thymidine, exposed to 20, 50, 100, 200 and 300r X-rays. Autoradiographs are prepared at various times after irradiation.—The post-DNA synthetic period (prophase) in the cell cycle is remarkably resistant to radiation, whereas the DNA synthetic period and the pre-DNA synthetic period, including the mitotic stages from metaphase through telophase and the portion of interphase preceding DNA synthesis, are extremely sensitive. Spermatogonia lethally hit by radiation die when they come to a definite stage in the mitotic cycle. The most remarkable finding is that the mitotic stage where cell death occurs is different in the three types of spermatogonia. It corresponds to metaphase in type B, to early prophase in intermediate, and to late interphase in type A spermatogonia.—These findings bear some possible implications as to the interpretation of the radiation response of mouse spermatogonia. The longer duration of the X-ray-sensitive, DNA-synthetic period and the shorter duration of the X-ray-resistant, post-DNA synthetic period in intermediate and type B spermatogonia in comparison to type A spermatogonia can possibly account for the greater X-ray sensitivity of intermediate and type B cells. The existence in the type A population of four-cell generations each having different durations of X-ray-sensitive and of X-ray-resistant periods offers a possible explanation of the heterogeneity of the radiation survival curves of these cells.

Morgan, Walter, and William Kohlmeyer, South Dakota State College, Brookings, S.D.: *Transmissibility of double oviducts in Aves.*—Genetic transmission of double oviducts, or persistent right oviducts, in fowl was first reported in an inbred line of Rhode Island Reds in 1957. Although the incidence is high (90%), egg production within this line has suffered from continual inbreeding. In an effort to incorporate the double oviduct condition into hens with higher productivity, outcrosses to unrelated stocks were made.—Original classifications were based upon autopsy findings. Since 1958 live hens have been externally classified upon the basis of manipulated appearance. When using the latter criterion, only those hens with positively identified right oviducal openings have been included as carriers in our data. A preponderance of hens with one oviducal opening, from crosses involving F_2 and backcross matings, indicates that the abnormal persistency is not transmitted as a simple Mendelian character. Correlated responses related to productivity and adult body weight will be considered.

MUELLER, JOAN O., and M. R. IRWIN, University of Wisconsin, Madison, Wis.: *Transferrin variation in pigeons and doves.*— Sera of *Columba livia* were examined by vertical starch gel electrophoresis. Three patterns of transferrins (iron-binding serum proteins, identified by Fe-59 autoradiography) were found and appeared to be genetically controlled. The two presumed homozygous types showed three transferrin zones and the heterozygous type showed four. The migration of heterozygous type sera was indistinguishable from that of a synthetic mixture of equal parts of the two different homozygous sera. The classification of 25 offspring from seven families representing five of the six possible mating combinations revealed no exceptions to the hypothesis that the three patterns are controlled by two codominant alleles.—Sera of two individual *C. guinea* showed distinct patterns which resembled one homozygous and the heterozygous serum patterns of *C. livia*, but differed from *livia* in the migration rate of the transferrins. Tests were made of the sera of four offspring from an interspecific mating between a heterozygous *guinea* and a heterozygous *livia*. The serum proteins of three of the offspring resembled those of the *guinea* parent; the fourth was like a mixture of the serum proteins of the two parents. These results are explicable on the assumption that there are two codominant alleles in *C. guinea* which are homologous to the two codominant alleles in *C. livia*.—Sera from several species of Streptopelia (dove) have been examined and similar phenomena have been observed. Individual variation was apparent within the majority of these species, and most of the species could be differentiated from one another.

MUKERJEE, DEBDAS, and H. P. RILEY, University of Kentucky, Lexington, Ky.: *Karyotype analysis of Haworthia species and varieties with special reference to the satellite chromosomes.* —Chromosomes were studied in six species and some of their varieties of the genus Haworthia (family Liliaceae, tribe Aloineae). Eight long and six short chromosomes constitute the karyotype of *H. attenuata*, *H. attenuata* var. *caespitosa**, an undetermined variety of *H. hybrida*, *H. planifolia* var. *typica*, *H.* sp. of the Tortuosae Section, *H. tortuosa*, *H. tortuosa* var. *major**, *H. tortuosa* var. aff. *torletta**, *H. tortuosa* var. *pseudorigida*, *H. tortuosa* var. *curta**, *H. reinwardtii* var. *tenuis**, and *H.* sp. of the Rigidae Section. In *H. planifolia* var. *typica* satellites are present on the short arm of one pair of long chromosomes and in *H.* sp. of the Rigidae Section, satellites are on the long arm of one pair of long chromosomes. In *H. attenuata* var. *caespitosa* two pairs of long chromosomes have satellites on the long arm but one chromosome of one of these pairs also has a satellite on the short arm, perhaps as the result of a translocation. No satellites have been found on chromosomes of any of the other plants. The idiograms of all the species or varieties are more or less different. Species or varieties apparently not previously studied are indicated by an asterisk. (This study was supported in part by Grant G–13220 of the National Science Foundation.)

MÜLLER, ILSE, and A. P. JAMES, Atomic Energy of Canada Limited, Chalk River, Ontario: *The influence of genetic background on the expression of radiation-induced mutations affecting a quantitative character.*—The effect of random ultraviolet-induced mutations on growth rate was studied in three diploid strains of the yeast *S. cerevisiae*. These strains differed in origin, in degree of heterozygosity, and in rate of cell division. The phenotypic effect of mutation was estimated from statistical comparisons of the distributions of growth rates of the descendant clones from irradiated and unirradiated cells. The induced mutations produced a positive shift (i.e., to a higher growth rate) in the fastest growing strain, a negative shift in the intermediate strain, and no detectable effect on the slowest strain. Presumably, similar spectra of mutant genes in the three strains interact with the respective genomes to produce distributions of phenotypes that are characteristic of the particular strain. This emphasizes the importance of epistasis in the expression of mutations affecting a quantitative character.

MUNDKUR, B., Institute of Cellular Biology, Univ. of Connecticut, Storrs, Conn.: *Submicroscopic morphology of the nucleus of Saccharomyces during tetrad formation.*—In frozen-dried preparations, the nucleus of Saccharomyces is at no time during division differentiated into

structures of dimensions clearly resolvable with the light microscope. At the level of the electron microscope, selective staining of nucleic acids reveals an organization of particles (about 80 Å) which have previously been shown to be DNA-protein in nature. In addition, there occurs in the nucleus, a hitherto undescribed body—a "peripheral cluster"—which assumes during tetrad formation stages, a form different from that of its equivalent in vegetatively dividing nuclei. The vesicular nature of the nucleus is maintained throughout the course of tetrad formation, though morphological variations are detectable at different stages, particularly in regard to the behavior of the peripheral cluster. The uniqueness of these nuclei resides in their display of classical genetical devices such as recombinations and first and second division segregations. The submicroscopic organization of the basophilia of both nucleus and cytoplasm during the entire course of presporulation and sporulation will be illustrated by means of lantern slides, together with a brief discussion of cytogenetical implications. (Work done in the Dept. of Anatomy, University of Chicago, during the tenure of a Special Research Fellowship from the National Cancer Institute, U. S. Public Health Service).

MUNKRES, K. D., and R. MEGNET, Yale University, New Haven, Conn.: *Mutational alterations of malic acid dehydrogenase in Neurospora crassa.*—Mutants of Neurospora with apparently altered malic acid dehydrogenases have been obtained by a variation of the WOODWARD, DEZEEUW, and SRB filtration-selective plating procedure.—Emphasis has been directed toward the physicochemical characterization of the normal and mutant forms of the enzyme. Comparative studies in crude extracts suggest that mutant malic acid dehydrogenases differ from the normal by the following criteria: (1) reduced specific activity for the forward and reverse reactions, (2) increased thermolability, (3) decreased heats of activation, and (4) altered substrate affinity constants. Preliminary studies bearing on the molecular weight and polymeric states of highly purified normal enzyme, as revealed by sedimentation velocity analysis and starch electrophoresis, will be discussed.—Genetic studies are in progress. Heterocaryon analyses indicate the mutants, thus far obtained, are allelic but "functionally" dissimilar.

MURRAY, NOREEN E., Stanford University, Stanford, California: *Polarized recombination with the me-2 gene of Neurospora.*—Twelve alleles have been used to analyze intragenic recombination. Methionine independent progeny from interallelic crosses were classified with respect to the markers which were present on both sides of *me-2*. Among the methionine protorophs, one of the two classes with markers recombined was always in excess; conversely, when the markers entered the cross in the alternate phase, a similar excess was always found in the reciprocal recombinant class. The 12 *me-2* alleles were ordered on the basis of these asymmetries. The sequence was consistent with a map based on additive prototroph frequencies (total map length $>0.2\%$ prototrophs).—Pronounced asymmetries were found between the two parentally marked classes of prototrophs and these, also, were reversed when the markers entered the cross in the opposite phase. If these asymmetries reflect differences in frequencies of gene conversion, then of any pair of alleles which has yielded a prototroph frequency greater than 5×10^{-5}, the allele that is the more distal has the higher conversion frequency. Thus for three alleles in the order a, b, c, the conversion frequencies are $b > a$, $c > b$, and $c > a$, in appropriate crosses.—This geometrical distribution suggests a mechanical explanation, but does not distinguish between a one- or two-mechanism hypothesis. The results are explicable on a switch hypothesis if the *me-2* region is situated immediately distal to some discontinuity in the genetic material. This would impose a non randomness of switching and make it unlikely that the switch region could be proximal to, yet extend into the *me-2* region. (Supported by Public Health Service research grant E1462).

NAKADA, DAISUKE, and KEIKO YANAGISAWA, (Introduced by RUTH SAGER), Columbia University, New York: *Thymine starvation and metabolic activity.*—During thymine starvation of *Escherichia coli* 15 thymineless (t^-) or its derivatives, the viability decreases rapidly to about one percent or less, the amount of DNA stays constant and a remarkable synthesis of both RNA and protein takes place. Thus it has been assumed that thymine starvation causes an irreversible

death of bacteria due to nuclear damage.—The present paper presents some evidence to show the metabolically active status of the thymine starved cells: (1) thymine starved cells synthesize a remarkable amount of DNA upon addition of thymine, but this DNA synthesis does not affect the viability; (2) the ability of thymine starved bacteria to form β-galactosidase declines concomitantly with the decrease of viability when induction is carried out in the presence of an external energy source, but when induction is performed without external energy source the thymine starved cells retain their ability to form β-galactosidase; (3) under conditions of thymine starvation, created by 5-fluorodeoxyuridine, the transfer and expression of *Z* gene (structural gene for β-galactosidase) is possible upon mating of *E. coli* K-12 HfV*Z*+ × F- *Z*-.

NAKAMURA, KAZUO, (Introduced by C. S. GOWANS), Department of Botany, University of Missouri, Columbia, Missouri: *Preferential segregation in linkage group V of Neurospora crassa.* —During meiosis the mutant allele at the *ts* (tan spore) locus tends to go toward the base of the ascus much more often than expected on a random basis. Biases at the first-division spindle and at the second-division lower spindle are both significant, and the latter bias is significantly greater than the former. No bias has been detected at the second-division upper spindle. There appears, thus, to be a gradient of polarized segregation from the lower to the upper portions of the ascus, indicating that the causes of the phenomenon may be extrachromosomal as well as chromosomal. There is no correlation between movements of the mutant alleles in the lower and upper second-division spindles within a single ascus.—The degrees of bias resulting from different crosses involving different wild type stocks do not differ significantly from each other, even though these different crosses give distinctly different frequencies of second-division segregation for the locus. The indication, then, is that crossing over between the locus and its centromere is not a controlling factor in this case of preferential segregation.—Evidence is presented that the degree of bias is influenced by temperature in a nonlinear fashion, the bias being greatest at 18°C. and less at both higher and lower temperatures. No evidence for inequality between frequency of asymmetrical and symmetrical second-division segregation has been obtained. (Work carried out at Kyushu University, Fukuoka, Japan).

NELSON, OLIVER E., JR., Purdue University, Lafayette, Indiana: *The effect of heterozygosity for cytological aberrations on recombination within the Waxy locus of maize.*—The frequency of $+^{wx}$ recombinant pollen grains in the F_1, $wx^{90} \times wx^c$, has been estimated as 88 to 105 × 10^{-5} in various genetic backgrounds. It is, therefore, interesting to note the effect on recombination between these two alleles of two structural aberrations (Dp 9 and Rearr. 9). Each aberration when heterozygous markedly suppresses intergenic recombination on the short arm of chromosome 9 where the *Wx* locus lies. Dp 9 has a piece of chromosome 3 inserted into the short arm of chromosome 9 near the *Bz* locus. The suppressive effect on intergenic recombination may be due to a steric hindrance with pairing. In the cross $\frac{+ \text{ Dp } wx^{90} \; +}{sh\,N \quad wx^c \quad gl\,15}$, the frequency of $+^{wx}$ recombinants is reduced significantly below the frequency in the absence of Dp 9. In most cases, however, the reduction is not proportional to the reduction in crossing over between the outside markers *Sh* and *Gl 15*. Heterozygosity for MCCLINTOCK's Rearranged 9 (a complex rearrangement including the *Wx* locus) almost eliminates recombination between loci located within the rearrangement since single crossovers give rise to dicentric chromatids. In plants which are $\frac{\text{Rearr. 9 } wx^c}{\text{N 9 } wx^{90}}$, the mean $+^{wx}$ frequency is 26 × 10^{-5}, while $\frac{\text{N9 } wx^c}{\text{N9 } wx^{90}}$ plants within the same progeny have a mean $+^{wx}$ frequency of 111 × 10^{-5}. (Supported by a grant from the National Science Foundation.)

NONNENMACHER, JACQUELINE, (Introduced by ELLSWORTH C. DOUGHERTY, University of California, Berkeley), Laboratory of Comparative Biology, Kaiser Foundation Research Institute, Richmond, Calif.: *Autoradiographic studies with tritiated thymidine in a nematode (Caenorhabditis briggsae).* The uptake of H^3-thymidine by *C. briggsae* grown axenically in a meridic

medium (=chemically defined basal mixture + crude organic supplement) has been followed autoradiographically, by both section and smear, Kodak AR-10 stripping film being used. Incorporation, so far detected only in nuclei, has been studied in two ways: (1) by rearing larvae with H^3-thymidine present; (2) by transferring young adults reared in nonradioactive medium, to radioactive. If derived from mass culture, larvae thereafter grown in H^3-thymidine-containing medium yield adults labeled in all somatic nuclei, also intensely in most gonadal nuclei (exceptions: mature germ cell nuclei); but, with adults, also from mass culture, only gonadal and sometimes intestinal nuclei become radioactive (in some adults of a given batch *all* gut nuclei are labeled; in others, *none*). In striking contrast, *no* labeling is detected, by either method applied to worms originating from eggs laid in an unsupplemented medium by adults left 24 hours therein.—Under no circumstances have sperm, oocytes, or zygotes been found with autoradiographically demonstrable nuclei. Conversely, whenever nematodes with labeled somatic and gonadal nuclei are transferred to a nonradioactive medium, the label is lost in two days. These findings raise intriguing questions—e.g.: (1) does incorporation of H^3-thymidine here represent formation of "nongenetic" DNA? (2) If so, are we seeing an exaggeration of the rôle of such "DNA" consequent upon nematode *eutely* (=relative nuclear constancy)? Whatever the answers, it seems likely that thymidine incorporation so far observed in *C. briggsae* has been a function of nutritional environment. (Supported, in part, by a grant from the National Institutes of Health, United States Public Health Service [E-3469].)

Nordskog, A. W., Iowa State University, Ames, Iowa: *Regression in egg production in the domestic fowl when selection is suspended.*—In poultry populations which have undergone artificial selection for egg production over many generations a decline might be expected when selection is relaxed. This is because concentrated selection on a single fitness trait, such as egg production should lead to negative genetic correlations with other fitness components. A seven-year experiment involving two strains of Leghorns and one RI Red showed a decline in each strain. The experiment included about eight sires and 80 dams in each breeding group per year and involved a total of 4080 birds over the seven years. After correcting the data for yearly trends and for linear selection effects associated with the initial populations, the net decline in egg production rate due to suspension of selection was —1.69% per generation in Leghorn B, —1.16% in Leghorn G, and —.97% in RI Red P. The average decline over the three strains was —1.35% per generation that selection was suspended.

Oakberg, E. F., and E. Clark, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Survival of spermatogonia of the mouse at different X- and gamma-ray dose rates.*—Hybrid (101 × C3H) male mice, 12 weeks old, were exposed to X- and Cs^{137} gamma-rays at the following doses and dose rates: 250 kv X-rays, 86 and 9.3r/min, 11, 21, 100, 300 and 600r; gamma rays, 0.8r/min, 5, 11, 100, 300, 600r; gamma rays, 0.009r/min, 5, 11, 300, 600, 1000r; gamma rays, 0.001r/min, 100 and 300r. Mice were killed 72 hr after irradiation for all treatments, with an additional group killed five days after 300 and 600r for the two X-ray and the 0.8r/min gamma-ray dose rates. For each mouse, type A spermatogonia were counted in a sample of 5 μ tubule cross sections distributed on the basis of a previously determined frequency of tubule stages in controls, and expressed as experimental/control ratios. Depletion of type A spermatogonia was independent of dose rate in the 86 to 0.8r/min range. Survival was slightly higher at 0.009r/min, suggesting that this dose rate is marginal in allowing recovery from the kind of radiation damage that leads to cell death. At 0.001r/min, survival of spermatogonia was greatly enhanced. These results will be discussed in relation to mechanisms of cell killing and to mutation-rate studies in Type A spermatogonia over this dose range.

Perkins, David D., Barbara A. Bloom, and Merle Glassey, Department of Biological Sciences, Stanford University, Stanford, California: *Genetic data on some Neurospora translocations.*—Rearrangements have been sought that are homozygous fertile, and that involve breaks in well marked regions or possibly in heterochromatin. Among such translocations, some are pheno-

typically wild: T(47711) involves linkage group I (between *arg-3* and *sex*) and group V (between *inos* and *bis*). T(S1007) involves I distal to *sex* and VII at *me-7*. T(R2355) involves IV distal to *cot* and V near *inos*. In other cases, such translocations have not yet proved separable from the mutant phenotype with which they originated: T(17084) (*thi-1* phenotype) involves I at *thi-1* and VII near centromere. T(44105) (*thr-1* phenotype) involves VII at *thr-1*.—Segregants from crosses with these rearrangements (except 44105) are readily scored for the translocation by crossing them in petri dishes to standard testers. If the translocation is present, ascospores shot to the lid from perithecia of a test spot include about 50% that are defective (white). Tests of T(44105) must be scored by opening perithecia, because defective ascospores are usually not projected.—Highly fertile standard strains of fluffy genotype have been selected for use as protoperithecial parents in scoring translocations (and in sex tests). These are technically advantageous because conidial scatter is minimized and ascospore shooting is not impeded or obscured. (Supported by Public Health Service research grant E1462.)

PETERSON, H. M., Botany Department, University of Illinois, Urbana, Illinois: *A case of spurious high negative interference in Drosophila melanogaster.*—A spontaneous aberration obtained in the course of studies at the Bar locus gave derivatives in recombination tests that simulated the high negative interference phenomenon reported in microorganisms.—"Single," "double," and "triple" exchanges were recovered in the 5.8 unit forked-carnation region of the X chromosome from an apparent "*f B odsy+ car*" strand in heterozygotes with a stock *f+ B+ odsy car+* chromosome.—The "*f B odsy+ car*" strand was shown by further genetic tests and cytological analysis to carry the segment from *B* to the right of *odsy* and *fu* (left of *car*) as a tandem serial duplication, cytologically the salivary regions 16A through 17E. Genetically the strand carries *f* (*B+ odsy+*) (*B odsy*) *car*; heterozygotes with a normal *odsy* strand behaved as trisomes for the region in producing about the same number of single crossover derivatives from each of three pairing situations: (1) left member of the duplication with homologue, giving six *f + odsy +* and four *f + + +*, (2) right member with homologue, giving 28 *+ + odsy car*, and (3) left member with right member, giving nine *f + odsy car* and one *f + + car*, among a total population of about 5,000 *B+* strands. The third type represents intrachromosomal exchange, a mechanism suggested to explain noncrossover derivatives from A^b alleles in maize and from Bar in Drosophila.—Evidence from this aberration not only confirms the occurrence of intrachromosomal crossing over within a tandem, serial duplication, but also suggests a type of cytological aberration which must be ruled out in cases where high negative interference is apparently operating.

PETERSON, H. M., and J. R. LAUGHNAN, Botany Department, University of Illinois, Urbana, Illinois: *Nonrecombinant derivatives at the Bar locus in Drosophila melanogaster.*—Studies involving the Bar duplication gave 32 spontaneous changes which were nonrecombinants for close markers in a population of over a quarter-million. Included were four changes from *B* to *BB*, 20 from *B* to *B+*, one from *B* to an intermediate allele, six from *BB* to *B*, and one from *BB* to *B+*.—Genetic and cytological analysis established that nine of the 32 changes were aberrations: five deficiencies (of different extent), one inversion, one duplication, and two others with low viability. Three other changes were genetically normal but cytologically identical with the parent Bar allele, and presumably represented mutations. Each of these twelve derivatives was shown to be different from the others, and they are considered to be the product of random, spontaneous events.—The largest class of changes, however, including 20 of the 32 derivatives, gave no genetic evidence of aberrations and the 14 that have been analyzed so far showed a cytological change corresponding to the change in phenotype. Since these changes involved the loss or addition only of 16A regions of the salivary map, they can not be explained as random events, nor are mutation, an activator-dissociator mechanism, misreplication of segments, or multiple exchanges likely explanations. Rather, the derivatives are considered to result from intrachromosomal exchanges between the paired, homologous members of the Bar duplication. This mechanism, suggested to account for similar occurrences at the *A* locus in maize, provides for the addition or loss of a member without marker recombination.

PIPKIN, S. B., The Gorgas Memorial Laboratory, Panama, R. de P.: *Relationships within the victoria species group, subgenus Pholadoris, genus Drosophila (Drosophilidae, Diptera).*—The victoria species group includes the Middle East Drosophila species *lebanonensis*, *pattersoni*, and *stonei*; also *D. nitens* from Italy; *D. victoria* from Palm Springs, California, an undescribed species from Tucson, Arizona, and an undescribed species from northern Arizona and Utah. Two members of the group, *D. lebanonensis* and the Tucson species, are polymorphic for light and dark mesonotum color, the polymorphism in *lebanonensis* depending on a single pair of alleles. *D. pattersoni* is a yellow species; the remaining are black, differing slightly in shininess and body size. A study of hybridization within the species group shows that the primary isolating mechanism has been the insemination reaction (studied originally by PATTERSON in the *repleta* group), whereby sperm are hampered and delayed by uterine secretions in reaching seminal and ventral receptacles and also egg laying is retarded. Hybrid death occurs at various stages between egg and pupa in most of the interspecies crosses, but imagines hatch in quantity in three cases. Hybrid females from the cross of *pattersoni* females and *stonei* males are fertile with *pattersoni* males but sterile with hybrid males and *stonei* males. Hybrid imagines from the cross of *pattersoni* females and the Tucson species are sterile *inter se* and with each parent species. However, reciprocal crosses made between *D. lebanonensis* and the northern Arizona and Utah species yield hybrids, fertile *inter se* and with each parent species. Nevertheless, the insemination reaction functions in retarding egg laying and causing the cross to be difficult to effect. When equal numbers of males and virgin females of *lebanonensis* and males and virgin females of the northern Arizona-Utah species are put together in laboratory bottles, both hybrid and pure species progeny result. Morphological studies of the male and female terminalia show minor differences only among members of the species group. The present study describes the unnamed species.

POPP, R. A., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Inheritance of different serum esterase patterns among inbred strains of mice.*—Mouse serum has several esterases that can be separated by zone electrophoresis in starch gels (MARKERT and HUNTER, J. Histochem. Cytochem., 7:42, 1959). Using such techniques, the patterns of serum esterases of C57BL/Cum and C57L/R1 mice differ from the serum esterase patterns of AKR/Up, RF/Up, 101/Cum, C_3H/Cum, BALB/cJ and SEC/R1 mice. Serum esterase patterns (zymograms) of the former and latter groups of mice differ as regards the number of esterases appearing near the albumin fraction on starch gel preparations. Zymograms of serum of C57BL and C57L mice reveal one band of esterase activity near albumin; however, two esterases appear near albumin in serum preparations of the latter group of mice. Sera of F_1 hybrids of (C57BL $\times$ 101)F_1, (C57BL $\times$ BALB/c)F_1 and (C57BL $\times$ SEC)F_1 mice have two bands of esterase activity in the albumin region of the starch gel, although the esterase band of slower electrophoretic mobility stains less intensely than on zymograms containing 101, BALB/c or SEC serum. Distinction of each of the parental and F_1 hybrid types can be made, however, by means of photometric assays (GOMORI, J. Lab. Clin. Med., 42:445, 1956). Such analyses indicate that the enzyme level in sera of F_1 hybrids is intermediate between that of either parental strain. Preliminary data of F_2 and BC_1 progeny are consistent with the idea that the variant esterase patterns are controlled by different alleles at a single locus.

POULSON, D. F., and B. SAKAGUCHI, Yale University, New Haven, Conn.: *Hereditary infections in Drosophila.*—The maternally transmitted "sex-ratio" condition, SR, of certain Drosophila species can be artificially transferred into normal strains of the same species and, in some cases, into other species, e.g. *equinoxialis* SR into *willistoni* (MALOGOLOWKIN); and we have transferred SR from existing strains in *willistoni*, *nebulosa*, and *equinoxialis* into *melanogaster*. The conditions of establishment, stabilization, and persistence of SR in the new hosts are under study. Progeny tests and clonal analysis show that genotypes both of host and SR agent are of utmost importance. Since SR females of these three species are characterized by the presence in the hemolymph of spirochetes which are absent in normal strains (POULSON and SAKAGUCHI, 1960, Anat. Record, 138, 376; MALOGOLOWKIN, 1960, Proc. 1st Cong. Zool. Brasil) there is strong evi-

dence that the SR condition is a special expression of a host-parasite relationship between fly and spirochete. Analysis of both intra- and interspecific transfers of spirochetes has provided evidence of persistent latent infections without differential effect on the sexes, but with variable egg, larval, or pupal mortality. Some apparently normal strains of flies carry such latent or attenuated infections.—Such infections are probably widespread in these species in nature.—The only case of SR which has so far resisted transfer is that originally studied in detail by MAGNI in *D. bifasciata*. This also differs from most others in its marked temperature sensitivity. Using a different approach to the nature of the *bifasciata* SR problem spirochetes from a *willistoni* SR strain were introduced into normal females of *bifasciata* in whose hemolymph they multiplied. Most of the progeny of such injected females died as larvae or pupae, but the survivors (at 25°C) consisted of both sexes in equal numbers, all carrying spirochetes in their hemolymph. A second generation at 25°C gave the same picture. The third generation was divided into two groups, those kept at 25°C with the above results, and those kept at 20°C in which the progeny was almost wholly female with only an occasional male just as in *bifasciata* SR. These results support the view that the maternally transmitted agent of *bifasciata* SR is basically of the same nature as those in the other species of Drosophila, representing an infection of long standing which has become integrated as a paragenetic element in natural populations of that species. (Supported by National Science Foundation Grants 6017 and 14747.)

RAI, K. S., and G. B. CRAIG, University of Notre Dame, Notre Dame, Ind.: *A study of the karyotypes of some mosquitoes.*—The morphology of mitotic chromosomes was studied from brain tissues of fourth instar larvae of five species of Aedes (*A. aegypti*, *A. albopictus*, *A. atropalpus*, *A. stimulans*, *A. vexans*) and one species each of Corethera, Anopheles and Culex. Studies were made from squash preparations stained with Breland's acetolactic orcein.—Although the diploid chromosome number of all species examined except Corethera ($2n = 8$) is six, there are significant cytological differences between some of them. Previously unobserved differences have been found among the chromosome pairs in *Aedes aegypti*. One of the pairs is small and the other two are relatively larger. The small pair (here designated chromosome I) and one of the larger pairs (chromosome III) are metacentric. The other large pair (chromosome II) is submetacentric, usually with a 4:3 arm length ratio.—Among the other Aedes the chromosomes of *A. atropalpus* closely resemble those of *A. aegypti*. However, each resting nucleus of *atropalpus* shows two conspicuous Feulgen-positive particles at opposite poles. The other three species of Aedes and *Culex pipiens pipiens* cannot yet be separated on cytological criteria. All of these possess three pairs of metacentric chromosomes with two large and one somewhat smaller pair.—*Anopheles quadrimaculatus* has three pairs of metacentric chromosomes, two of which are much larger than the minute third pair. The resting nuclei have usually one deeply staining particle.—In *Corethera sp.*, in addition to three elongate pairs, there is a fourth dot-shaped pair of chromosomes and a prominent deeply staining particle. (Supported in part by Atomic Energy Commission Research Contract AT[11-1]-38.)

RATTY, FRANK J., San Diego State College, San Diego, California: *A comparison of mutation rates in male and female pre- and postmeiotic germ cells of Drosophila.*—This study has attempted to determine whether the mutation rates of the yellow (*y*), white (*w*), split (*spl*), and forked (*f*) loci contained in the white-mottled-4 (w^{m4}) chromosome are comparable in male and female germ cells. The work reported herein is based upon the examination of 244,106 F_1 of both sexes derived from irradiated w^{m4} males and 172,000 F_1 of both sexes derived from irradiated w^{m4} females. White-mottled-4 parents were mated to *y w spl f* flies following exposure to 3,000r and the progeny were scored for the occurrence of viable and lethal mutations. The parents were subcultured to new media every three days for a maximum of 24 days. A comparison of the mutation rates indicates an excess of *y*, *w* and *w-N* mutants in ova whereas more *N*(*w*+) mutants were found in sperm. A comparable number of *f* mutants was found among the progeny of irradiated males and females. In the period 4–12 days after irradiation, an excess of all types of mutations was found in spermatocytes. However, subsequent to this period no mutants were found

following the examination of 35,873 females derived from irradiated males, whereas ten *y*, one *w*, one *w-N* and ten *f* mutants were recovered from a comparable number of progeny of irradiated w^{m4} females up to 24 days subsequent to the time of irradiation. It would appear that both viable and lethal mutations are either produced more readily in premeiotic female than male germ cells or they are transmitted with greater facility. (This work was supported by a grant from the National Science Foundation, Contract G-6391).

REDDY, G. M., and E. H. COE, University of Missouri and U. S. Department of Agriculture, Columbia, Mo.: *Complementary interaction and gene action sequence in anthocyanin synthesis in maize.*—The synthesis of anthocyanin pigment in the aleurone tissue of maize is controlled by several factors, including A_1, A_2, C_1, C_2, and *R*. It has been established that these genes must be present in dominant condition for the production of anthocyanin. If any one of these genes is recessive, or if dominant C^I is present, colorless aleurone results. It also has been established that Bz_1, Bz_2, *Pr*, and *In* determine the intensity and nature of anthocyanin.—"Active" (22- to 25-day old) aleurone tissues of self-fertilized testers, each having only one gene recessive, were pressed together in pairs, in all possible combinations. Pieces of distinguishable size were used, and the pairs were placed in test tubes on agar. Complementary interaction resulted in pigment synthesis in all combinations, and was unidirectional in each case (the "donor" was unpigmented and the colorless "receiver" developed pigment). This indicates that some diffusible substrate from the donor is used by the receiver to synthesize pigment. Since the receiver tester carries the dominant gene lacking in the donor, as well as all subsequent factors, the factor recessive in the receiver but present in the donor must act first in the sequence. However, *in*-tester behaves either as strong or weak donor, and it is suggested that its modifying action may follow the action of *R*. Bz_1, on the other hand, acts more or less as a uniform donor when combined with other testers, which suggests that its action may follow all the others. Interestingly, C^I gives pigment with c_1 tester, presumably its own allele, and also with the rest of the testers, suggesting that, at the least, the inhibitory action of C^I precedes the action of C_1. These observations extend and provide independent confirmation of previous investigations, and establish the following gene-controlled stepwise synthesis of anthocyanin:

C^I——C_1——*R*——(*In*)——A_1——A_2——(Bz_1) - - - - - - Anthocyanin — Further analysis of the complementary interaction of C^I and c_1 tester, investigation of the sequential action of C_2, *Pr*, and Bz_2, and some additional chemical studies are in progress.

RÉDEI, G., and LOTTI M. STEINITZ-SEARS, University of Missouri, Columbia, Mo.: *X-ray mutants with high selective advantage in Arabidopsis.*—Among 1474 X-ray treated seed progenies, four different mutations have been obtained at three different loci which affect the length of the vegetative period of the plants. Under long-day conditions the wild type develops visible flower primordia in 10–12 days. The mutants, however, require up to 60 days for the completion of the same developmental stage. The longer life cycle is accompanied by an increase in plant height up to almost three times, the production of up to 20 times as much dry matter, and up to ten times as many seeds. All four mutations are simple recessives under long-day conditions, but one becomes dominant under short days. Transmission is normal on both male and female sides. The most extreme mutant was checked cytologically. The five pairs of chromosomes invariably form bivalents with 1–2 chiasmata at the first meiotic division. The chromosomes are very small, the longest being the nucleolar chromosome (about $2/\mu$ at anaphase I). The 10 to 12 heterochromatic chromocenters (prochromosomes) in the interkinetic nuclei lie almost always at the nucleolar periphery. In mitotic divisions there is a striking juxtaposition of homologues (somatic pairing). The autogamous populations were grown through successive generations starting with F_1 under constant illumination in the greenhouse in an area of fixed size, without other artificial selection. The three mutants tested showed a high selective advantage. After the completion of ten generations the wild phenotype was reduced to ten percent in one population and to less than one percent in the other two. In natural habitats the most prevalent late ecotypes are controlled by several dominant or semidominant genes, apparently nonallelic to the induced muta-

tions. Mutations affecting photoperiodic response may have been important in the adaptation of the species. Speculations are possible as to whether an early or a late type was the ancestor.

RODERICK, THOMAS H., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *The genetics of variation of sensitivity to X-irradiation in mice.*—Two-way selection of adult animals for resistance to chronic X-irradiation has been effected from a four-way cross. Selection was successful for the criterion of length of survival under 100r/day (realized $h^2 = +.41$ after 4 generations) but unsuccessful for the criterion 400r/day (realized $h^2 = -.01$ after five generations). Approximately 20 adult animals of both sexes from 26 inbred strains were subjected to the same criterion (100r/day). Significant strain differences in length of survival were found. Males were more resistant and also more variable in their resistance than females. A *positive* genetic correlation was found between length of survival and adult body weight in the selected lines. The body weights of the animals of the high selected line were about twice those of the low line. A general positive within line, within generation correlation existed between the two variables which was presumably the genetic basis for the association after selection for length of survival. On the contrary in the inbred strain study, a *negative* genetic correlation existed between mean strain length of survival and mean strain body weight. No consistent within strain correlation was observed. (Supported by Atomic Energy Commission Contract AT[30-1]–1979 and National Institutes of Health Grant RG 7249.)

ROTHEIM, MINNA B., University of Rochester, Rochester, N.Y.: *A complex genetic locus concerned with streptomycin resistance in Pneumococcus.*—A genetic analysis, by means of DNA-mediated transformations, of 11 independently-derived spontaneous mutations to streptomycin-resistance in Pneumococcus has shown that all are either allelic (occur at the same site) or closely-linked (occur at different sites) markers on the same molecule of transforming DNA. Examination of the genetic map constructed by this analysis leads to several conclusions. First, there are more mutations than sites. Second, the distribution among the sites can be observed to follow a pattern; there is a definite tendency for mutations conferring similar levels of resistance to occur at the same site. However, not all the mutations at one site are identical since it has been possible to differentiate between them by means of a modifier gene.—There is also a genetic factor (or factors), termed *depressor*, linked to the streptomycin-resistance markers, which by itself has no effect on the level of resistance but which apparently decreases the frequency of genetic integration of most of the streptomycin-resistance markers to which it is linked. The *depressor* appears to have reverted simultaneously with the reversion of one of the streptomycin-resistance markers. Furthermore, experimental evidence makes it unlikely that the *depressor* existed in the strains prior to the occurrence of the streptomycin-resistance mutations. It is suggested that the *depressor* is a rearrangement of the genetic material, adjacent to the sites of the streptomycin-resistance mutations, which has occurred simultaneously with the mutation to resistance.

RUDKIN, G. T., and JACK SCHULTZ, The Institute for Cancer Research, Philadelphia, Pa.: *Disproportionate synthesis of DNA in polytene chromosome regions in Drosophila melanogaster.*—The autocatalytic synthesis of DNA in nuclei usually follows a geometric progression in which the common ratio is two. This rule breaks down in certain regions of polytene chromosomes of the higher Diptera during the last endoreduplication before metamorphosis. It has been suggested that these localized deviations are the result of (1) asynchrony of DNA synthesis in the different chromosome parts, each part following the doubling rule in the long run, or (2) aberrant phenomena presaging cytolysis of the giant cells. It will be shown that heterochromatic and euchromatic regions in salivary gland chromosomes of Drosophila cannot both be replicated according to the same geometric progression throughout development, and that the period during which they differ in this regard must be very early, probably in embryonic stages.—DNA was determined at the end of the larval period by photometry in the ultraviolet (257 mμ) after ribonuclease treatment. The ratio of DNA in heterochromatin to DNA in euchromatin was

found to be much higher in ganglion cell metaphase X chromosomes (1:2) than in a salivary gland X chromosome (1:50), as would be expected from cytological observations. In a separate experiment (outlined in Science, 132: 1499, 1960) the amount of DNA synthesized during the first day of larval life, marked by feeding H^3-thymidine, was compared with the total amount synthesized by the end of the larval period, measured in the same salivary gland chromosomes by photometry. The ratio of early synthesized DNA (grain count) to total DNA at the end of larval life (UV absorbance) was found to be the same in both types of chromatin (heterochromatin and euchromatin : 3.0 and 2.8 in an X; 2.5 and 2.2 in a 2R chromosome of a male larva). The simplest interpretation of these data is that DNA synthesis had approximated a geometric progression throughout the larval period, maintaining the disproportion established during earlier nuclear development. Differences in the ratio of early to total DNA synthesis between very short segments of euchromatin suggest that nonproportionality may be encountered in the larval period, but the significance of these differences has not been established.

Russell, Liane Brauch, and Clyde L. Saylors, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *Spontaneous and induced abnormal sex-chromosome number in the mouse.*—The spontaneous frequency of X^MO continues to be relatively high (0.7%; varying from 0.1 to 1.7% in various stocks). X^MX^PY is rare (0.02%). $0X^P$ and X^MX^MY have still not been observed. We have, therefore, concluded that, in the mouse, spontaneous nondisjunction at the first meiotic division is rare in both sexes. The relatively high frequency of X^MO could be due to other types of sex-chromosome loss in male gametogenesis, or to loss of paternal X or Y following fertilization.—Significant increases in X0 frequency have been achieved by irradiation at various stages. Results of one experiment, already reported in part (Russell, Science 1961), indicate that the early portion of the interval between fertilization and the first cleavage is particularly sensitive: 100r yielded 5.18% sex-chromosome loss (control frequency: 0.97%). Both X^MO and $0X^P$ animals (a type that has not occurred spontaneously) were found; 0Y is presumably lethal. No indications of mosaicism have been obtained, either phenotypically or on genetic tests. There is both genetic and cytological evidence that entire sex chromosomes, rather than pieces, are lost. During a later period in the fertilization-to-first-cleavage interval, sensitivity both to X0 induction and to killing decreases.—Prefertilization irradiation of sperm significantly increases the frequence of loss of the paternal sex chromosome: 600r yielded 1.6%; control frequency, 0.2%. However, sperm is apparently less sensitive to X0 induction than is the early postfertilization (pronuclear) stage.—Attempts are being made to induce nondisjunction in meiotic divisions of both males and females.

Russell, W. L., and Elizabeth M. Kelly, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee: *Mutation frequency in mice exposed to radiation of intermediate dose rate.*—Specific-locus mutation frequency is being determined for spermatogonia of mice irradiated with 600r (Cs^{137} source) at 0.8r/minute. This intensity is approximately the geometric mean of the two intensities (0.009r/min and 90r/min) used in our original discovery of the intensity effect. To date, ten presumed mutations have been observed in 27,840 offspring. This agrees closely with the mutation frequency obtained in the earlier experiments at the low radiation intensity of 0.009r/minute. It is significantly ($P = 0.001$) below the mutation frequency obtained from the same total dose of X-rays delivered at 90r/minute. It is also significantly ($P = 0.026$) below the mutation frequency obtained in a recent 600r gamma-ray (Co^{60} source) experiment in which the intensity was 24r/minute.—The last comparison shows that the range over which most, or all, of the originally observed intensity effect occurs has been narrowed down from a 10,000-fold to a 30-fold difference in dose rates.—In the 0.8r/min experiment, the length of the temporary sterile period following irradiation was no shorter than that observed at the highest dose rate of 90r/min, indicating similar amounts of spermatogonial killing. This was confirmed by accurate measurement of spermatogonial survival, described in the accompanying abstract by Oakberg and Clark. Thus, reducing the dose rate from 90r/min to 0.8r/min lowers mutation frequency, but does not reduce killing in the cell population in which the mutations occur. The dose-rate effect on mutation is, therefore, independent of cell killing.

SAND, SEAWARD A., Connecticut Agricultural Experiment Station, New Haven, Conn.: *Alien chromosome addition races in Nicotiana.*—*N. longiflora* (n=10) has been crossed with *N. sanderae* (n=9). These distinct species, both in section Alatae, produce a completely sterile F_1 hybrid. Colchicine treatment has given the fertile amphidiploid, LLSS (n=19). Backcrosses of LLSS to *longiflora* (LL) and to *sanderae* (SS) are successful with difficulty, and the respective sesquidiploids, LLS and SSL, have been grown. SSL has been completely sterile. However, LLS gives seed when pollinated with LL. The resulting progeny segregate dramatically in a discontinuous distribution. This has been evaluated by measurements of flower dimensions.—The simplest expectation is that these progeny contain *longiflora* cytoplasm and the diploid *longiflora* complement (LL) plus various numbers (0–9) of extra *sanderae* chromosomes. Complications may be anticipated due to interspecific exchanges between chromosomes. These may be minimal, however, as is suggested by (1) chromosomal sterility of the F_1 hybrid, (2) little segregation of the amphidiploid, (3) meiotic behavior of the sesquidiploid (10II, 9I), and (4) duplication of discrete phenotypes in the segregating progenies.—Phenotypes deviating from *longiflora* have been selected throughout the range of variation. These are being recurrently backcrossed to *longiflora*, attempting to isolate the nine single *sanderae* chromosome addition types ($LL + I_S$). Presently two families have behaved as expected for an alien chromosome addition, giving longiflora-type plants plus one offtype which duplicates the female parent. One of these has a longer corolla tube than *longiflora*, presumably due to one particular extra chromosome from shorter-tubed *sanderae*.

SANDERS, B. G., and J. E. WRIGHT, The Pennsylvania State University, University Park, Pa.: *Inheritance of a major blood group system in rainbow trout.*—Erythrocyte antigens R_1 and R_2 have been identified in rainbow trout, *Salmo gairdneri*, by means of the agglutination test utilizing reagents prepared from antisera produced in rabbits. These reagents have been designated R1 and R2. A pair of alleles, R^1 and R^2, determine these antigens, such that the three genotypes R^1R^1, R^1R^2, R^2R^2 determine the phenotypes R-1, R-1-2, and R-2, respectively.—Crosses in all possible combinations among these types produced fingerling progeny of the types and proportions expected.—Seven randomly breeding populations have been sampled and gene frequency analyses have been carried out. Only two of these populations show a fit to expected frequencies of populations in equilibrium. In the remainder there is a significant deficiency of the heterozygous class. However, inbred lines that have been sampled show no fixation of these alleles. There is an indication of different allelic frequencies in different geographic populations.—Numerous tests indicate that the two antigens are specific for rainbow trout in that blood of brook, brown, and lake trout do not react with the two reagents.—The R2 reagent differentiates blood of heterozygotes and homozygotes by difference in rapidity of agglutination.

SARKISSIAN, IGOR V., S. S. SHAH, (Introduced by M. M. GREEN), University of California, Davis, California: *Differential responses of two barley genotypes to chloramphenicol.* Seedlings of S_{13} generation of hooded (*KK*) and awned (*kk*) barley in Atlas 46 background were grown five days in contact with 0.01% chloramphenicol. Seventeen free amino acids were identified by paper chromatography of alcoholic extracts of the seedlings. Quantitative determinations of the individual amino acids were carried out with the following results: in the seedlings of both genotypes, serine, threonine, lysine, arginine, tyrosine, and proline were not affected by the treatment. Asparagine, glutamine, histidine, valine, phenylalanine, and the leucines showed a highly significant decrease in the treated seedlings of both genotypes. Aspartic and glutamic acids decreased slightly in the treated seedlings, while α-alanine and γ-aminobutyric acid increased.—Highly significant genotypic differences were observed with glycine, asparagine, α-alanine, and γ-aminobutyric acid. Awned exceeded hooded in glycine by 20%. Asparagine content was greater in *kk* than in *KK*. α-Alanine and γ-aminobutyric acid were present in greater amounts in both treated and control seedlings of *KK* and in *kk*. Highly significant genotype-treatment interaction was observed with asparagine, α-alanine, and γ-aminobutyric acid. Asparagine decreased slightly in the treated seedlings of the awned and decreased significantly

in the treated hooded. α-Alanine increased significantly in the treated *KK*, increasing slightly in the *kk*. γ-Aminobutyric acid was increased by the chloramphenicol treatment of both genotypes, the increase being significantly higher in the hooded.—Percent total nitrogen was not affected by the chloramphenicol treatment. Percent nitrogen in tissue from which amino acids had been extracted was greater in controls of both genotypes. The results suggest a possible biochemical differentiation of two essentially isogenic lines differing at one locus.

SCHAIBLE, ROBERT, and JOHN W. GOWEN, Iowa State University, Ames, Iowa: *A new dwarf mouse.*—A dwarf type was discovered segregating in a line of extreme nonagouti ($a^e a^e$) mice derived from a cross between Goodale's 'Large' and a stock descended from an irradiation experiment (HOLLANDER and GOWEN, J. Heredity 47:221, 1956). Although phenotypically similar to the pituitary dwarf (*dw*), the new type proved not to be allelic and is therefore named Ames dwarf. Growth retardation after the first week results in retention of juvenile body proportions, myxedema, and accumulation of fat. Weight at two months averages only 12 gm. Reproductive organs remain immature; no dwarfs have reproduced without treatment. Four male and two female dwarfs, initially ranging in age from 24–54 days, were given daily intraperitoneal injections (0.5 mg) of bovine growth hormone (supplied by DR. R. T. HILL, National Institutes of Health.) Treatment was continued until the mice appeared nearly normal and growth had ceased (usually after six weeks). By that time the males weighed 22–24 gm; all four subsequently sired litters. The females weighed 18 and 21 gm but did not reproduce. The Ames dwarf in general behaves as a recessive character. However anomalous sex ratios, appearance of a tortoise-shell dwarf male (*To* usually lethal in male), and evidence of linkage with markers Rex and waved-2 indicate that a reciprocal translocation between the X sex chromosome and VII autosome may be involved. (This work has received assistance from Contract No. AT[11–1]107 from the Atomic Energy Commission. Journal Paper No. J-4112 of the Iowa Agricultural and Home Economics Experiment Station, Ames. Project Nos. 1180 and 1187.)

SCHNEIDER, IMOGENE, (Introduced by JANICE B. SPOFFORD), The University of Chicago, Chicago, Ill.: *Modification of V-type position effects in Drosophila virilis.*—A series of six translocations, each of which evokes a position effect of the peach locus in *D. virilis* were studied to determine whether the parental source of R(*pe*+) has any influence on the amount of pigmentation in the heterozygous variegated offspring. Utilizing paper chromatography, quantitative measurements were made on the drosopterins and a number of other pteridines, some of which presumably act as intermediates in the synthetic pathway leading to the formation of the red pigments. Measurements were made on the eyes of both male and female offspring, and on the testes sheaths of the males.—Without exception, heterozygous variegated offspring are more fully pigmented if R(*pe*+) is transmitted by a homozygous female parent in contrast to a heterozygous female, homozygous or heterozygous male parent. Homozygous male parents usually produce more fully pigmented offspring than do heterozygous male parents. Variegated offspring receiving R(*pe*+) from heterozygous mothers often have more pigment than the corresponding offspring receiving R(*pe*+) from heterozygous fathers. These statements are true only so far as the visible pigments (drosopterins) are concerned. The other pteridines, in contrast, show no consistent pattern as to the parental source of R(*pe*+).—With respect to one of the Y;5 translocations studied, the presence of a Y chromosome in the genome of the mother suppressed the phenotypic expression of mottling in her male offspring, even though they did not receive this chromosome. Paralleling this finding is the fact that the addition of an extra Y chromosome to the genome of the same peach-mottled stock led to an even greater suppression of variegation; a finding inconsistent with the generalization that extra heterochromatin enhances the extent of mottling in V-type position effects associated with genes located in heterochromatin. (This research was supported by the Atomic Energy Commission, Contract No. AT[11–1]–431).

SCHWINCK, ILSE, Abt. Entwicklungsphysiologie, Max-Planck-Institut für Tierzucht und Tierernährung, Mariensee über Neustadt/Rübenberge, Germany: *Quantitative studies on the*

temperature dependence of drosopterin formation and of semilethality in the mutant rosy of Drosophila melanogaster.—The main pleiotropic characters of the mutant rosy (*ry*) are : (1) reduced amount of drosopterins (red eye pigments) and their nonautonomous formation as demonstrated in transplantation experiments, (2) semilethality in the late pupal stage and during imaginal moult, (3) aberrant morphology and function of the Malpighian tubes, and (4) no xanthine dehydrogenase activity and no accumulation of isoxanthopterin and uric acid. In course of studies on regulating mechanism in the manifestation of the pleiotropic pattern the strains ry^1, ry^2, $v;ry^2$, $cn;ry^2$ and four isogenic strains of $cn;ry^2$ were bred at 18° C and 25° C. Visual classification of the eye color as well as quantitative determination of the drosopterins by fluorometric measurement on paper chromatograms with the Zeiss-Spectralphotometer on the one hand, and data on lethality in late pupal stage on the other hand, demonstrate a positive correlation between increasing amounts of drosopterins in flies cultured at 18° C, to a level of about 200% of the amount found in 25° C cultures, and an increasing percentage of flies emerging from pupae. About 85% emerge in 18° C cultures compared with about 50% in 25° C cultures. The question of temperature effect on Malpighian tubes and xanthine dehydrogenase is still under investigation. (Supported by a grant from the U.S. Public Health Service.)

Sears, E. R., and W. Q. Loegering, Crops Research Division, U. S. Department of Agriculture, Columbia, Mo., and Beltsville, Md.: *A pollen-killing gene in wheat.*—A gene (*Ki*) located on chromosome 6B (X) of the Chinese Spring variety of common wheat results in the early abortion of *ki* pollen in *Ki ki* plants. That it also leads to abortion of *Ki ki* microspores in *Ki Ki ki* plants is indicated by the nearly 50% aborted pollen produced by *Ki Ki ki* trisomics. When hemizygous (in monosomic 6B), it is not lethal to pollen lacking the *Ki* locus, for some 20-chromosome (nulli-6B) pollen functions. Linkage of *ki* with a dominant gene (*Sr*) for stem-rust resistance in Timstein and other varieties distorts the expected 3:1 ratio to one ranging from 2.4:1 to 1.0:1, depending on the variety used (Sears and Rodenhiser 1948; Knott 1959; Luig 1960). In Chinese × Timstein a linkage value of about 10.5% is indicated. Leaf-rust resistance (*Lr*) of Pawnee must also be linked with *ki*, for while it segregated 3:1 in crosses with Timstein (Heyne and Johnston 1954), it gave a 9:7 ratio following a cross to Chinese Spring (Heyne and Livers 1953). Linkage values of 11.5% between *ki* and *Lr* and 21.7% between *Sr* and *Lr* were indicated. Thus *ki* appears to lie about half way between *Sr* and *Lr*. Only the varieties Chinese Spring and Mentana have thus far been shown to carry *Ki*, but any variety derived by selfing a cross with a *Ki Ki* variety will be *Ki Ki*. In a cross-pollinating species a mutant such as *Ki* would quickly replace *ki*.

Seto, Frank, Berea College, Berea, Ky.: *A comparative study of second chromosome lethals from wild and laboratory populations of Drosophila melanogaster.*—Samples of flies from three sources were examined for their content of recessive lethal factors. Two of the samples were collected in 1959 and 1960 from wild populations of *Drosophila melanogaster* in the Berea, Kentucky, area and a third was obtained from a laboratory population initiated with flies from part of the 1959 collection. The frequency of second chromosome recessive lethals in the different samples were: wild population (1959), 31 percent; wild population (1960), 37 percent and laboratory population (1960), 23 percent. The intrapopulation allelism rates for lethals in these three populations were 1 percent, 1 percent and 3 percent respectively. The interpopulation allelism rate between lethals of wild population (1959) and the related laboratory population (1960) was a low .28 percent.—The period of action was roughly determined for the various lethals and they were classified as egg, egg-larval, larval, larval-pupal, and pupal lethals. The ontogenetic distributions of lethality for the three series of lethals were compared and there was no significant difference. It appears that the genetic variability in wild populations, as measured by the lethal frequency, allelism rate and frequency of lethals manifesting their effects at different stages, is relatively constant from year to year. Although there was a reduction in genetic variability in the laboratory population which is due probably to the decrease in population size, there was no change in the relative frequency of recessive lethals affecting different stages in development. (Supported by NSF grant G106–99).

SHAW, R. F., University of Virginia School of Medicine, Charlottesville, Virginia: *The time course of the aging process in Drosophila.*—If flies are kept for a time at 25° and then put in an environment of 29°, they have a shorter life span than when kept continuously at 25°. The exact length of life depends upon the time at which the change of temperature is made.—Supposing senility and death of the organism to result from some physicochemical state that changes continuously with time, it is possible to see whether the time course of aging follows the usual expression for chemical processes. This is a function in which the rate of reaction is proportional to a power of the concentration of reactants. If the time function is of such a type it can be shown that life span in the temperature-change experiments will have a linear relationship to the time at which temperature is changed, and also that the slope of the line is predictable as a simple function of life span at 25° and at 29°.—These predictions have been tested using recently emerged males. Shell vials containing food were stocked with 12 flies. The number remaining alive was observed every 3½ days when the food was changed. The time at which only four remained was taken as the end point, or measure of life span.—The findings agree with the expectation of linearity. A statistical comparison of the theoretical with the observed slope is being made, and the result will be reported.

SHREFFLER, D. C., and R. D. OWEN, California Institute of Technology, Pasadena, Calif.: *Association between a heritable serum variant and the Histocompatibility-2 locus in the mouse.*—Using the OUCHTERLONY technique of double diffusion in agar, with rabbit antiserum against mouse serum globulins, 13 inbred lines have been classified into two groups on the basis of a wide difference in the position of the precipitin band formed by one component of the serum. The sera of F_1 progeny from crosses between differing lines form the band in an intermediate position. Tests of F_2 and backcross progeny have established that the parental and F_1 phenotypes segregate as discrete types and in the frequencies expected with single gene control without dominance.—Five inbred lines having one serum type (CBA, C3H, AKR, RF, Flexed) all carry the *H-2*^{k} allele, while the eight lines of the other serum type (A/He, A/Jax, C57Bl/6, DBA/1, DBA/2, BALB/c, WB, WC) have other *H-2* alleles. This suggested a possible association between the two traits. Among segregating progeny classified for both the serum antigen and *H-2* red cell specificity, the two traits segregated together in 88 of 91 mice tested. The three exceptional cases may have resulted from recombination or from nongenetic variations. Among the non-*H-2*^{k} lines, smaller differences in precipitin band position between C57Bl/6 (*H-2*^{b}) and the other lines (*H-2*^{a}, *H-2*^{d}, *H-2*^{q}), and between sexes within lines have also been observed.—Preliminary studies to characterize the serum factor antigenically suggest that the difference is quantitative. Analyses by agar and starch gel immunoelectrophoresis indicate that the component has the mobility of an alpha globulin, and that it is probably of high molecular weight.

SHULT, E. E., and C. C. LINDEGREN, Southern Illinois University, Carbondale, Ill.: *Affinity in Saccharomyces.*—In prior work, preferential segregation was observed between a chromosome carrying a gene (*CU-2*) controlling copper resistance, and chromosome V. This effect was repeated in 15 crosses and was most intense with the genes *UR-3* and *CH-1* which bound the centromere of chromosome V, but becoming progressively weaker with four linearly ordered markers distal to *CH-1*. Subsequent intra-ascal crosses indicated that preferential property resided on the chromosomes being segregated. Failure to duplicate this effect with copper mutants, obtained from MORTIMER and HAWTHORNE, led to the discovery that their locus, *CU-1*, was nonallelic with the former. However, the locus *CU-1* exhibited the ratio 1:18:21 for PD:NPD:T with the gene *AR-4*. Two intra-ascal matings obtained from one of the 18 NPD tetrads exhibited ordinary linkage consistent with both previous data and the intra-ascal results described above. A third intra-ascal mating obtained from the single PD tetrad indicated that this tetrad is due to either double exchange or the destruction of the affinity capacity.

SILAGI, SELMA, (Introduced by L. C. DUNN), Department of Zoology, Columbia University, N. Y.: *Partial complementation between lethal alleles at the T locus of the house mouse.*—The

complexity of the *T* locus in the mouse, at which many alleles have been described, makes it favorable material for relating the central function of the gene to phenotypic effects of the alleles. The three earliest acting lethals at this locus, which kill embryos from the morula stage (preimplantation) to the early egg cylinder (just after implantation) were combined in compounds and found to produce 30% of the expected number of newborn compounds, always retarded in development. There is therefore complementation between alleles with respect to the process which results in lethality, but never perfect restoration of normality. Most of the embryos die between 11 and 12 days, the placentation period, but show onset of abnormality at seven days, when primitive streak and mesoderm are forming. Variation in degree of abnormality among compound embryos is great, even within the same litter. The most common defects are abnormal forebrains and varying degrees of anophthalmia. Only 35% of compounds born have lived to be weaned. None obviously abnormal survive the neonatal period. The females are fertile, the males thus far tested have been sterile. The results are consistent with the hypothesis that each allele supplies something not produced by the other, so that an interaction product may be formed in sufficient quantity to carry the embryo well beyond the lethal periods of the homozygotes, but below a critical threshold which determines whether fairly normal development may proceed. Death occurs at placentation if the embryos are too retarded to connect with the umbilical circulation.

SOKOLOFF, A., Biological Laboratory, Cold Spring Harbor, New York. *Preliminary studies on the effect of X-ray on Tribolium imagoes.*—Three day old imagoes of *T. castaneum* and *T. confusum* were exposed to X-ray dosages of 1,500, 3,000 and 6,000r administered in 2.5, 5 and 10 min, respectively. The various studies lead to the following conclusions: (1) Productivity (measured in terms of F_1 adults produced) of *T. castaneum* irradiated females $\times$ nonirradiated males was greater for the 1500r experimentals than for the controls; 3000r resulted in 75% and 6000r in 100% drop in productivity (assuming productivity of controls to be 100%). (2) The lethal effect of the high dosages of irradiation takes place at the egg stage. Mortality of larvae emerging from zygotes of irradiated males $\times$ nonirradiated females or from zygotes of the reciprocal cross is not greater than of larvae from unirradiated controls. (3) Dominant-lethal frequency generally increases with heavier irradiation until at 6000r 99% or more of the eggs fail to hatch. (4) Males of *T. castaneum* appear to be more sensitive to X-rays than females, and *T. confusum* males more sensitive than *T. castaneum* males: productivity of the latter was only 11.5% of the controls at 3000r, and 0.5% at 6000r; for *T. confusum* corresponding values were 8.0% and 0.2% of the controls. (5) The drop in productivity persists for at least one generation after exposure: *T. castaneum* controls produced 95 F_2 per female; F_1 of 3000r irradiated males $\times$ nonirradiated females produced only 63 F_2 per female. Comparable values for *T. confusum* were 90 F_2 per female for the controls and 32 for the experimentals. (6) No sex-linked lethals were obtained. One visible mutant (pink eye) was probably induced by X-rays.

SONNEBORN, T. M., and R. V. DIPPELL, Indiana University, Bloomington, Ind.: *The modes of replication of cortical organization in Paramecium aurelia, syngen 4.*—Four structurally diverse kinds of cells (normals, doubles, and two different incomplete doubles) have been shown to reproduce true to type independently of relevant differences in genes, nuclear differentiation, nuclear size or free-flowing endoplasm. The diversities of structure are localized in the cell cortex, and different parts reproduce at fission in different ways. (1) Certain parts, such as mouth and gullet, arise only beside and under the influence of pre-existing mouth and gullet. (2) Other parts arise from nonidentical precursors; e.g., the anterior suture arises from the cleavage furrow immediately to the left of the oral meridian and independently of the presence of a mouth or gullet. (3) The diverse patterns of kineties to the right and left of the oral meridian, result from differential growth of pre-existing kineties. (4) Other parts, such as the cell anus (cytopyge), arise wherever the posterior right and left kinety patterns are juxtaposed. Such mechanisms result in the perpetuation, during reproduction, of additions or losses of parts of the cortical organization. The specific substances utilized in the formation of these cortical parts are doubtless

derived directly or indirectly from gene action, as may also be the specific mechanisms in the fission processes. Nevertheless, where the substances go and how they are organized into the visible landmarks of the cell cortex are determined by the pre-existing organization of the cortex itself, as proved by the genetic analysis above. (Supported by grant American Cancer E 81 B Sonneborn.)

SONNEBORN, T. M., and R. V. DIPPELL, Indiana University, Bloomington, Ind.: *Self-reproducing differences in the cortical organization in Paramecium aurelia, syngen 4.*—It has long been known that double paramecia with 180° between corresponding structures, reproduce true to type through both fissions and conjugation with each other or with singles. Incomplete doubles of two sorts also reproduce true to type. One sort has two oral meridians, each complete with anterior suture, mouth, gullet, posterior suture and cytopyge, flanked by the typically diverse patterns of kineties to the right and left, but the two oral meridians are only about 90° apart. There is but one row of contractile vacuoles, but the contractile vacuole pores (and presumably contractile vacuoles) are paired laterally instead of single. Frequently, a third cytopyge appears between those on the two oral meridians, where the left posterior kinety pattern of one meridian abuts upon the right posterior kinety pattern of the other. The second sort of incomplete double lacks a mouth and gullet on one oral meridian but retains the sutures, cytopyge and characteristic kinety patterns to the right and left of this meridian; the other oral meridian, 180° away, is complete. Reproduction of both sorts of incomplete doubles shows that cortical segments and even parts of incomplete cortical segments reproduce true to type. The origin of these cortical variations and the genetic analysis of some of them show that these hereditary cortical differences are self-determining and not due to genic, nuclear or endoplasmic differences. (Supported by grant American Cancer #E 81 B Sonneborn.)

SORIANO, J. D., University of the Philippines, Quezon City, Philippines: *Chiasma frequency and chromosomal segments involved in interchanges in rice.*—Examination of five varieties of the *indica* type of *Oryza sativa* L. gave 12 bivalents at metaphase I. The bivalents were either ring bivalents, one chiasma in each arm, or rod bivalents, one chiasma is one arm. The number of chiasma ranged from 14 to 22 per cell with a chiasma frequency ranging from 1.4 to 1.7 per bivalent or a mean of 1.6.—These data were used in determining relative length of chromosomal segments involved in interchange complexes in irradiated populations. In a variety with a chiasma frequency of 1.5, six ring bivalents and six rod bivalents, the occurrence of all the ring bivalents, four rod bivalents and a ring of four chromosomes at metaphase I indicates two relatively long segments included in the interchange configuration.—A maximum of two ring interchanges and one chain interchange involving one half of the genome per cell was obtained in highly sterile lines after dry-seed irradiation.

SPIELER, RICHARD A., (Introduced by WILLIAM K. BAKER), University of Chicago, Chicago, Ill.: *Nondisjunction involving the maroon-like and rosy mutants of Drosophila melanogaster.*—Recently DR. JACK L. HUBBY called my attention to maroon-like, a mutant strain of *Drosophila melanogaster* in which he had observed a high incidence of primary nondisjunction of the X chromosomes. Females of this stock produce primary exceptional sons at a frequency of 1.2% of all male progeny, compared with the commonly quoted frequency of about 0.08% in wild type. Exceptional daughters, however, occur in about the same proportion as in wild type. A stock containing another mutant allele, *ma-l*^{bz}, shows normal disjunction, as do stocks of *y*^{2}*ma-l*^{bz} and *Bx*^{3}*ma-l*. Certain heterozygous combinations, for example, *ma-l*/*y*^{2}*ma-l*^{bz} and even *y*^{2}*ma-l*^{bz}/*Bx*^{3}*ma-l*, yield exceptional sons at a frequency of 0.6–0.7%; the frequency of exceptional daughters is normal. Another stock, *ma-l;st*, produces exceptional sons at about 2% and exceptional daughters at 1%. Heterozygotes such as *ma-l*/+ and *y*^{2}*ma-l*^{bz}/+ give the normal frequency of nondisjunction. This indicates that the phenomenon may be related to the *ma-l* locus itself, variously modified in different genetic backgrounds, and not to the production of exceptional males arising from four-strand double exchange within undetected inversions.—A third chromosome

mutant, rosy, produces 1.3% exceptional sons and 0.9% exceptional daughters. Its allele ry^2 does not show an abnormal incidence of nondisjunction.—Since both *ma-l* and *ry* block the production of xanthine dehydrogenase, these findings raise the interesting question of the possible role of pteridine metabolism in chromosome behavior. (Supported by U. S. Public Health Service Grant RG-7428.)

SPRAGUE, LUCIAN M., Bureau of Commercial Fisheries, Biological Laboratory, Honolulu, Hawaii: *Erythrocyte antigens of the oceanic skipjack (Katsuwonus pelamis) recognized by phytoagglutinins.*—Saline extracts of leguminous seeds often have the property of agglutinating vertebrate erythrocytes. While many species of legumes yield extracts which agglutinate the erythrocytes of skipjack, five species, *Maackia amurensis, Virgilia capensis, Caragana arborescens, Glycine max,* and *Phaseolus vulgaris,* produce fluids which will recognize individual differences between skipjack bloods by agglutination procedures. Maackia extracts agglutinate all skipjack tested but following absorption by selected cells a fraction remains which exhibits an identical reaction pattern in comparison with Virgilia extracts.—In tests of 642 skipjack with extracts of the above seeds, four distinct patterns of reaction have been observed. Virgilia and Caragana permit the recognition of three phenotypes. Bloods agglutinated by Virgilia fail to react with Caragana. Bloods agglutinated by Caragana fail to react with Virgilia. Some bloods fail to react with either extract.—The patterns detected by Glycine and Phaseolus are apparently independent of one another and the Virgilia and Caragana patterns.—The relative proportion of the phenotypes recognized by Virgilia-Caragana and by Glycine are significantly related to the geographic origin of the samples.—A continuing study of the relative proportion of phenotypes in samples from different geographic localities in the central North and South Pacific Ocean is being made in an attempt to relate racial groups with marine ecology. Acknowledgment is made to PROFESSOR CHARLES COTTERMAN for the initial samples of seed.

SPIESS, E. B., MARY ROWAN CAPENOS, and ROBERT B. HELLING, University of Pittsburgh, Pittsburgh, Pa.: *Linkage analysis of lethals on second and third chromosomes from a laboratory population of Drosophila melanogaster.*—In the process of extracting homozygous chromosomes II and III from a laboratory population of *D. melanogaster* which had been maintained for more than 170 generations at 25°C (WALLACE's Oregon-R control #3, 1949, population) with intent to study the effects of recombination on viability, the following lethals were isolated: 18 out of 103 second chromosomes tested and 21 out of 89 third chromosomes tested. Lethals balanced over dominant markers (*CyL* and Ubx^{130} for the IInd and IIIrd chromosomes respectively) were intercrossed for allelism and also mapped by using appropriate markers (*al pr stw c sp, al dp b pr c px sp,* and rucuca). The distribution of lethal loci is non random on chromosome II. On chromosome II eleven different loci are arranged mostly in the left arm (one subterminal, two in mid-2L, and the remainder clustered around the centromere). The subterminal (l_7) and mid-2L (l_1) lethals were represented four and three times respectively. At least three lethal chromosomes contained no less than two lethal loci. On chromosome III the five lethal loci tested so far are arranged in mid-arm regions of both sides and no lethals have so far been located near the centromere. Allelism tests revealed that one lethal was represented five times, one four times, and two twice. These facts suggest that many lethals are in much higher frequency than could be maintained by mutation pressure alone and that they may confer heterosis in heterozygous condition. The finding of several multilocus lethal chromosomes also implies a selective advantage for such combinations or for those combinations which engender multilocus lethals by crossing over. (Work done under Contract AT[30-1]–1775, U. S. Atomic Energy Commission; third author National Science Foundation Cooperative Fellows.)

STADLER, JANICE and JOHN W. GOWEN, Iowa State University, Ames, Iowa: *Viability in the mouse as affected by cobalt-60 irradiation "continuous" through ten generations.*—Extrapolations of the results from one species to another or of single generations tests on one species have frequently been utilized to guess at what would happen to future generations were the irradiation to be continued over succeeding generations of progenies. Experiments to test what actually happens

have been few. To furnish data on this problem we have been exposing mice of several strains to eight different dosages of irradiation from cobalt-60 over periods averaging 22 hours a day throughout their lifetimes and that of their descendants for each succeeding generation. Continuous dosages of 12r per day have resulted in sterility and the loss of the germ line. Dosages of 3r per day have given occasional litters which have allowed the continuation of the germ line in successive generations. Dosages of 1–2r per day have allowed sufficient reproduction to continue the germ lines until now progenies are available for the 11th generation. Contrary to the expectations of many, the long term continuous irradiation of the germ plasms over these generations, has allowed phenotypically vigorous mice to be produced within these highly inbred strains. During the course of these generations no mutations have been observed although we do have other experiments in which they have occurred. Sterility genes have not become all important factors in reproduction over the ten generation period. (This work has received assistance from Contract AT[11-1]107 from the Atomic Energy Commission. Journal Paper No. J-4113 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1180, 1187.)

STEFFENSEN, DALE M., Department of Botany, University of Illinois, Urbana, Ill.: *An objective method for calculating chromosome structure and its application to Tradescantia chromosomes.*—A number of parameters can be used to delimit and to determine chromosome structure. It is no longer appropriate to speculate on this subject for too much evidence is known about the chemical and physical nature of chromosomes. The method provides an empirical solution to the problem at hand. Each step can be challenged, corrected but not ignored. Five parameters are outlined below accompanied by values for Tradescantia chromosomes in parentheses along with notations about the methods of measurement: (1) *Chromosome diameter* (0.39 μ at late interphase) by light and electron microscopy and X-ray diffraction, (2) *Chromosome length* (single chromosome corrected for "major" coiling = 182 μ at interphase, n = 6) by light microscope, (3) *Number of DNA molecules per micron of chromosome* ($3 \times 64 = 192$) by physical-chemical methods and electron microscopy of DNA and nucleoprotein, (4) *Amount DNA per 2n nucleus* (4.5 to 7.0×10^{-12} gm, derived indirectly) chemical analysis, photometric methods and "target theory," (5) *Molecular orientation and degree of coiling for molecules and fibrils* (orientation of molecules and fibrils is longitudinal, submicroscopic coiling exists but the degree has yet to be determined) by numerous physical and optical methods. It follows that parameters (1), (2), and (3) can be used to estimate the DNA content per nucleus in parameter (4). The number of molecules multiplied by total chromosome length gives 2.1×10^5 DNA molecules ($182 \times 6 \times 192$) times 1.0×10^{-17} gm (wt. of single molecule using Avagadro's number) gives 2.0×10^{-12} gm DNA per 2 "C" nucleus. An expected coiling factor for DNA-protein fibrils in parameter (5) would increase this latter calculation accordingly. The various values indicate most strongly that Tradescantia chromosomes are multistranded. The possibility of their being single-stranded DNA is essentially eliminated.

STINSON, H. T., JR., Conn. Agr. Exp. Sta., New Haven, Conn.: *Nuclear influence on chloroplast multiplication in Oenothera.*—Reciprocal crosses between the seven paired, alethal species *Oenothera hookeri* and *O. argillicola* produce inviable chlorophyll-deficient F_1 hybrids; *hookeri* chloroplasts (*H*) and *argillicola* chloroplasts (*A*) are defective when associated with the F_1 nucleus (*ha*). Viable interspecific hybrids are obtained, however, when the plastids of the female parent are replaced with *O. parviflora* plastids (*P*), but some individuals from these crosses are variegated. The cross *hookeri* with *parviflora* plastids (*Phh*) ♀ × *argillicola* (*Aaa*) ♂, for example, yields plants with green (*Pha*) and yellow sectors (*Aha*), the latter resulting from the male transmission of *argillicola* plastids. Flowers arising from the *Aha* yellow sectors were backcrossed with pollen from *O. hookeri* (*Hhh*) in an attempt to place an *hh* nucleus with *A* plastids. This cross gave solid yellow, yellow-green variegated, and green plants. In test crosses the green plants behaved as though they contained exclusively *hookeri* plastids like those in the male parent. The cross *Aha* ♀ × *Phh* ♂ also gave some fully green plants, but these plants contained typical *parvi-*

flora plastids. Apparently, nuclei approaching the *hh* condition prevent or inhibit multiplication of *A* plastids contributed by the egg, but, as expected, allow multiplication of *H* and *P* plastids transmitted via the pollen. The *A* plastids are thus lost from the growing point early in development and are replaced by either *H* or *P* plastids. In the F_1 nuclear stage (*ha*), *A* plastids are at no such disadvantage, as best shown by the cross *Aaa* ♀ × *Phh* ♂ which produces no wholly green plants (*Pha*) and few variegated plants.

STRAUSS, B. S., University of Chicago, Chicago, Ill.: *Mutagenic and nonmutagenic effects of alkyl methanesulfonates.*—Incubation of lag phase cells of *E. coli*, strain WP-2, in buffer at 48°C after treatment with ethyl methanesulfonate (EMS) and thorough washing results in a large increase in the number and frequency of revertants. This effect is not obtained with methyl methanesulfonate (MMS). MMS is mutagenic but produces a high proportion of small colony revertants in contrast to EMS induced revertants which are uniform and of the large colony type. The presence of dinitrophenol or chloramphenicol during buffer incubation after EMS has no effect.—A sensitive test for EMS, MMS and ethyl sulfate has been devised based on reaction with pyridine to give a nonvolatile N-alkyl pyridine salt with an absorption maximum at 259 mμ. The posttreatment effect occurs in the absence of detectable free EMS.—Treated cells lost their sensitivity to heat after incubation in growth medium. Addition of chloramphenicol to growth medium prolonged the sensitive period.—Exponential cells are rapidly killed by incubation in buffer at 48°C after treatment with methanesulfonates. MMS is more effective in this posttreatment reaction than EMS. Lag cells are resistant.—Similar effects have been obtained with another *try* mutant of B/r. A *try* mutant of K-12(*td-2*) is resistant.—The results support the idea that EMS forms an ethylated guanine within the DNA which decomposes to yield an apurinic acid; methyl guanine derivatives are more stable. It is suggested that a chloramphenicol sensitive process restores the genome to a stable state. (Work supported by a grant from the National Institute of Health.)

STRELZOFF, EUNICE, (Introduced by F. J. RYAN), Columbia University, New York: *Induced mutation and DNA replication.—E. coli 15 t⁻* has been used to study 5-bromouracil (5BU) incorporation into DNA in the absence of thymine. It was found that exponentially growing cells can increase their DNA fivefold upon resuspension into 5 BU medium. Macromolecular increase is linear and follows the series x, 2x, 3x, . . . nx where x is the amount of DNA initially present. Paper chromatography has shown that 5 BU is incorporated in place of thymine in newly synthesized DNA. Cell death proceeded during DNA synthesis in 5 BU at a constant rate with slightly less than half of the cells dying during the first DNA doubling.—Newly synthesized DNA characterized by total substitution of 5 BU for thymine was shown to be genetically functional by the use of *t⁻*-amino acid-requiring cells. The amino acid requirement was induced by the use of 2-aminopurine (2AP) or 5 BU. Synchronized cells were observed to revert from auxotrophy to prototrophy following a DNA doubling in 5 BU and resuspension into fresh medium. Reversions were also observed to take place when DNA increased in 5 BU medium in the absence of other macromolecular increase. This was made possible by the use of chloramphenicol or *t⁻*-amino acid-uracil-requiring auxotrophs.—Reverse mutations were studied at a number of sites for frequency of reversion, number of DNA replications required for phenotypic expression, and pattern of revertant increase with DNA increase. These results were compared with 2 AP-induced reversion at the same site. The time of appearance followed that predicted by FREESE's theory of transitional errors (J. Mol. Biol. 1, 87, 1959), and the pattern of increase made it possible to distinguish errors of incorporation from errors of replication and to tentatively identify the base pair at the mutant site. (Work supported by Public Health Service research training grant.)

SUZUKI, D. T., (Introduced by EILEEN SUTTON GERSH), University of Chicago, Chicago, Ill.: *Effects of X chromosome inversion heterozygotes and homozygotes on crossing over within the third chromosome of Drosophila melanogaster.*—OKSALA (1958) suggested a model for interchromosomal effects on crossing over in which the distal and proximal regions play an important

role in chromosome orientation at meiosis. A study of crossing over in inversion homozygtes offers a method for studying these regions without possible complications of loop formation. The inversions rst^3, y^4, y^{3P}, sc^4, and sc^8 have breakpoints in different distal and proximal spots and were tested as heterozygotes and homozygotes. Crossing over was studied in five regions of chromosome 3 with "res" markers.—All of the inversion heterozygotes increased the total map length relative to the control (X/X) length, the centromere and adjacent regions having the largest increases.—The map length in y^{3P} homozygotes was not different from the control value. Flies homozygous for sc^4 showed greatly increased crossing over in the centromere region (40%) and in the middle of 3R (20%), whereas sc^8 homozygotes had a significant but small increase (8–10%) in four regions and a large increase in the middle region of 3R (26%). Females homozygous for y^4 had increased values in all but the centromere region. The map length in rst^3/rst^3 females was increased only slightly.—These data along with similar studies being completed using sc^9 and w^{m4} inversions provide a test of certain of the assumptions on which OKSALA's model is based. (This research supported by the Atomic Energy Commission, Contract No. AT[11-1]–431.)

THOMPSON, P. E., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.: *The effect of asynapsis on mutability in the testis of Drosophila.*—In males of Drosophila, normal chromosomes paired with inverted chromosomes have shown higher rates of spontaneous and X-ray induced mutation than isogenic controls. This difference in mutability is most striking in meiotic stages, where its magnitude after irradiation implies a synergistic interaction of treatment and the genetic constitution. The effect is limited to those chromosomes in which structural heterozygosity exists.—Two alternative hypotheses toward an underlying mechanism of increased mutability have been advanced: an effect of stress or torsion associated with inversion-pairing configurations, and an effect of the partial asynapsis that must accompany structural heterozygosity. A test of the effect of asynapsis without torsion is made possible by the use of SM5, a complex intrachromosomal rearrangement having 13 inversion or transposition breaks, which does not pair appreciably with its homologue. Preliminary results indicate high mutability of the unsynapsed homologue of SM5 and suggest that homologous pairing itself is an important factor in mutation, presumably by some facilitation of repair.

VALENTINE, F. A., State University College of Forestry at Syracuse University, Syracuse, N.Y.: *Orange variegated pericarp in maize.*—Orange variegated pericarp (P^{ovov}), and uncommon *P* allele in maize, occurs as a rare mutant from ordinary variegated (P^{vv}). Eight independent mutations of P^{ovov} have been isolated, four from the Wisconsin variegated, two from a central Pennsylvania strain, and one in each of two other variegated stocks. Orange variegated phenotype conspicuously differs from variegated by the presence of a homogeneous orange-red background color between the prominent red stripes. The frequency of striping conditioned by each of two P^{ovov} alleles in inbred W23 background averages 40 percent less than the common variegated stock from which they arose. The mutational behavior of P^{ovov} parallels that of P^{vv}. The most common mutants among the progenies of orange medium variegateds are orange light variegated and self red. The orange light variegated phenotype exhibits a 64 percent reduction in the frequency of striping compared with orange medium variegated. A similar reduction in ground color is indicated by the statistically significant linear regression of ground color on frequency of striping in three independent tests. Results from a fourth test are comparable but are not statistically significant. Preliminary tests of several independent occurrences of two rare mutants from orange variegated, mutant white and a variegated-like phenotype, are discussed. The present evidence supports the hypothesis that orange variegated results from a mutation of the Modulator component of P^{vv}.

WEIR, J. A., University of Kansas, Lawrence, Kansas: *Sex ratio studies in mice.*—Intensive efforts to change the sex ratio of PHH (high sex ratio) and PHL (low sex ratio) lines of mice by nongenetic influences (feeding HCl or NaOH in the diet, mating early and late in the estrus

cycle, double inseminations, artificial inseminations) have served to re-emphasize the high degree of genetic control over sex ratio. Matings of PHH males to outbred females at the end of their estrus period and matings of PHH virgin females to PHH males sometimes results in a lowering of the sex ratio. PHL males rarely mate with females in late estrus. Natural double inseminations result in a surplus of unions of eggs and spermatozoa from animals of the same strain; also litters are larger than from single inseminations. Except as noted above, sex ratios are not influenced by the various kinds of matings.—Metabolic processes, under endocrine control seem to be responsible for lactic acid differences (PHH 3.2 ± 0.15 and PHL 5.1 ± 0.20 μ moles/ml lactic acid). There is no evidence for direct effects of *p*H on the sex ratio.—Matings to outbred ♀♀, K, produced litters as follows: K♀♀ × PHH♂♂, 52.6% ♂♂ (147 litters, 1,132 mice), K♀♀ × PHL♂♂ 39.0% ♂♂ (96 litters, 803 mice). The overriding influence of the male continues to be the most impressive feature of the sex ratio factor. (Work supported by U. S. Public Service Research Grant RG-7786.)

WHITING, P. W., SARAH B. CASPARI, and DORIS J. BUSH, University of Pennsylvania, Philadelphia, Pa.: *Marker genes and multiple alleles.*—The gene, defined as whatever segregates as a unit in meiosis, may contain many specific factors affecting different vital processes. Induced factor differences associated with a convenient marker may be selected and thus a considerable series of mutant alleles may be obtained. In Mormoniella two associated eye-color factors, *O* and *S*, in the "*R*" chromonemal region, mutate to oyster-white and to scarlet respectively. Oyster and scarlet have served as markers for much less frequent eye-color mutations and for numerous factor injuries causing crossover reduction, inviability, sterility and other defects. Whatever the physical basis—deletions, repeats, inversions—the great number of factor states found and subject to test may be simply the number of different ways in which the *R* region may be modified and still remain functional and transmissible. Dominant lethals probably occur but have not been noted. Presumed dominant steriles have been scored as single individuals. Homology of recessives, which is determined by complementation, may indicate that the same action path is affected. There is evidence that similar relationships obtain in three chromonemal regions other than *R* and it is probable that any chromonemal region with a suitable marker could be induced to respond in like manner. (Supported by U. S. Atomic Energy Commission Contract AT[30-1]–1471.)

WOODWARD, VAL W., Rice University, Houston, Texas: *Specific alterations of the complementation pattern of a pyr-3 mutant of Neurospora.*—Complementation patterns exhibited by large numbers of allelic mutants reflect aspects of chromosome structure as evidenced by colinearity between genetic and complementation maps. As a guide to structure, however, complementation has several shortcomings. One of these is the ambiguity between noncomplementation resulting from combinations of alleles unable to complement and noncomplementation resulting from phenomena extrinsic to the alleles in question.—Evidence of specific extrinsic factors affecting complementation was obtained in the following way: conidia of the *pyr-3* mutant, KS-23col, were irradiated with an LD-50 dose of UV prior to mixing with conidia of the noncomplementing strains KS-36alb and KS-43alb. Following irradiation, heterocaryon colonies were formed. Homocaryon strains were recovered from the heterocaryons and tested for altered complementation patterns. The KS-23col mutant recovered from the 23 + 43 heterocaryon was heterocaryon-positive with recovered KS-43alb, original 43alb, and the original 43 strain, but its complementation pattern with ten tester strains, including KS-36, was unchanged. KS-23col recovered from the 23 + 36 heterocaryon behaved similarly with the original KS-36 strain. Growth of the "new" heterocaryons approximated that of wild type. In each case the change was specific to the mutant with which the "induced" heterocaryon was formed.—The cross, recovered KS-23 × original KS-23, failed to yield prototrophs (5×10^6 ascospores). Segregation of the induced het-positive phenotype will be discussed. (Research supported by grant no. RG-5833[C2], National Institutes of Health.)

YERGANIAN, GEORGE, Children's Cancer Research Foundation and Harvard Medical School, Boston, Massachusetts: *Meiosis and X chromosome differentiation.*—The *Triheterosomic* scheme for sex determination stipulates the female to be X_1X_2 and the male, X_1Y. However, in the newborn Chinese hamster, oocytes have uniformly appearing sex (XX) bivalents which, accordingly, may be designated as X_1X_1. The SAT region of the heterochromatic long arm is distinctly separated from the proximal portion by a large nucleolus. The short arms pair entirely, as indicated by a chromomeric pattern, indistinguishable from other euchromatic (autosomal) segments. In order to facilitate homologous pairing of the X's, the short arm of the original X_2 had to differentiate from its entirely heterochromatic appearance (and function) to one of euchromatic potency, $X_2 \rightarrow X_1$, during oogonial or preoocyte I stages. Thus, all eggs contain an X_1 chromosome. During the counterpart stage of development of the sex bivalent in the male, a reciprocal differentiation occurs, i.e., $X_1 \rightarrow X_2$. The exceedingly prominent allocyclic appearance of the sex bivalent in the spermatocyte reflects this transformation. The strong "pairing" affinity of the Y chromosome with the transformed X_2 is due to the Y being "homologous" to the short arm of the X_2. The X_2 and Y chromosomes are physiologic counterparts and impart femininity or masculinity when combined, separately, with the neuter-sexed element, the X_1 chromosome. Thus, sperm bearing either an X_2 or Y chromosome determine sex following fertilization with eggs bearing only the X_1 chromosome.—X chromosome differentiation, i.e., $X_1 \rightarrow X_2$ and $X_2 \rightarrow X_1$, occurs during meiosis or haploidization. Anticipated changes in heteropyknotic relationships are fully reflected during the course of this unique series of events.

YOON, CHAI H., Boston College, Chestnut Hill, Mass.: *Linkage relationship of the waddler gene in mice with evidence for temperature effect upon crossing over.*—A neurological condition named waddler (gene symbol, *wd*) is a recessive trait in mice. Linkage experiments carried out with F_2 and backcross generations showed that the gene, *wd*, is linked with brown (gene symbol, *b*) and pintail (gene symbol, *Pt*) in linkage group VIII and the order of the genes is *Pt*, *b*, *wd*. The recombination frequency between *wd* and *b* was found to be 29.4% in the F_2 data and 32.5% in the backcross data, the χ^2 values for joint segregation in both cases being statistically significant at the 0.1% level. However, when the same experiment was carried out with a backcross generation in a room where the temperature, on the average, was about 10°C higher than the normal room temperature, the genes *wd* and *b* segregated as though they were independent, indicating a significant effect of the higher temperature upon the crossing over frequency in the area between *wd* and *b*. (Supported by a grant from the National Institutes of Health.)

GENETIC CONTROL OF THREE HAPTOGLOBINS IN PIGS[1]

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THE haemoglobin-binding proteins of serum (haptoglobins) were first identified by POLONOVSKI and JAYLE (1940). SMITHIES and WALKER (1955, 1956) identified three haptoglobin phenotypes in man under genic control of alleles Hp^1 and Hp^2. CONNELL and SMITHIES (1959) described a fourth phenotype designated haptoglobin type 2-1 (modified), but did not establish the genetic basis for this haptoglobin phenotype. Phenotypes in man having no haptoglobin were described by ALLISON, BLUMBERG and REES (1958). BLUMBERG (1960) and KRISTJANSSON (1960) observed one haptoglobin fraction in pig serum.

The purpose of this investigation was to obtain discrete separation of the pig haptoglobins and to determine the genetic mechanism in the inheritance of haptoglobin phenotypes in this species. It is uncertain if these haptoglobins are the same COHEN (1959) observed among inbred lines of Large White pigs.

MATERIALS AND METHODS

The population considered in this study included litters produced from matings of six Yorkshire sires to 52 Yorkshire and 20 Landrace dams and from matings of six Landrace sires to 33 Landrace and nine Yorkshire dams. Serum samples were prepared from all sires and dams and from a random sample of the progeny in each litter.

Approximately 100 mg/100 ml of porcine haemoglobin solution was added to each serum sample. The haemoglobin was prepared from cells thoroughly washed in 0.9 percent saline, lysed with distilled water and centrifuged to remove the stroma. The samples with added haemoglobin were then subjected to starch gel electrophoresis (SMITHIES 1959). The gels were made with 70 g hydrolysed starch (Connaught Medical Laboratories, Toronto) in 500 ml of a 0.061 M tris (hydroxymethyl) aminomethane–0.0039 M citric acid buffer solution. The electrode chambers contained a 0.3 M boric acid–0.05 M NaOH solution. Electrophoresis was carried out for 17 hours at 6.6 v/cm.

On completion of electrophoresis the gels were sliced and stained with benzidine reagent (200 ml distilled water, 0.4 g benzidine, 1.0 ml glacial acetic acid and 0.23 ml 30 percent H_2O_2) for two hours. The stained gels were then washed in running tap water for one hour after which each sample was typed. Gels were stored in water in a refrigerator.

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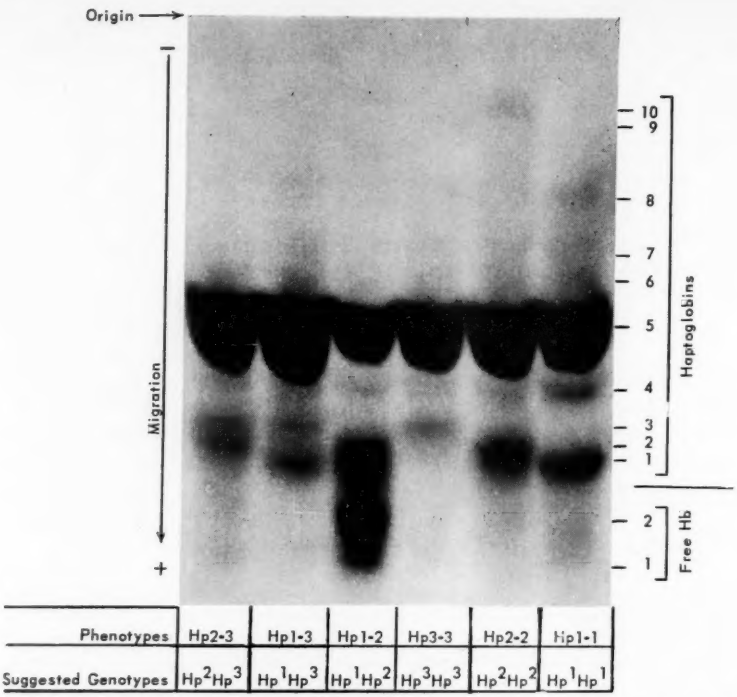

FIGURE 1.—Gel stained with benzidine reagent showing positions of various haemoglobin and haptoglobin fractions. Phenotypes and genotypes are indicated for the polymorphisms observed for haptoglobin fractions 1, 2 and 3.

RESULTS AND DISCUSSION

Figure 1 is a photograph of a gel showing the haptoglobin-haemoglobin complexes and free haemoglobin stained with benzidine. Since the bound haemoglobin is used merely to characterize these protein fractions as haptoglobins, the haptoglobin-haemoglobin complexes will be referred to as haptoglobins. No benzidine-positive bands were observed in samples devoid of haemoglobin and the haemoglobin preparation used showed only the benzidine-positive bands indicated as free haemoglobin 1 and 2.

The amount of haptoglobins 4 and 5 present varied considerably from sample to sample but no attempt was made to study this variation. Haptoglobins 6–10 were not considered in the present study because these fractions fade rapidly after staining.

The six phenotypes (designated as Hp 1-1, Hp 2-2, Hp 3-3, Hp 1-2, Hp 1-3 and Hp 2-3) involving polymorphism for haptoglobins 1, 2 and 3 are shown in Figure 1. Three phenotypes contain only one of the three haptoglobins while the remain-

ing three phenotypes represent the three possible combinations of two of the three proteins. None of the samples analysed contained all three haptoglobins nor was any devoid of all three haptoglobins.

The variation in density of haptoglobin fractions 1, 2 and 3 indicated in Figure 1 is due mainly to small differences in the amount of haemoglobin added to the samples. Any significant excess of Hb added to the serum resulted in a broadening of the haptoglobin-haemoglobin complex band and created difficulties in typing. This was particularly true in the case of the Hp 1-2 and Hp 2-3 phenotypes as the two bands became virtually one broad band if there was much excess Hb added.

In a review of genetically controlled protein polymorphisms, ALLISON (1959) found that "the commonest situation seems to be that in which allelic genes control the formation of closely related but distinct proteins, two of which are present in heterozygotes." This observation coupled with the distribution of progeny phenotypes for haptoglobins 1, 2 and 3 presented in Table 1 suggested

TABLE 1

Distribution of progeny phenotypes (observed/expected) from various mating classes and the results of χ^2 tests of their fit to the hypothesis that phenotype Hp 1–1 = Hp^1Hp^1, Hp 1–2 = Hp^1Hp^2, Hp 1–3 = Hp^1Hp^3, etc.

Matings	Progeny phenotypes*					P of χ^2
	Hp 1–1	Hp 1–2	Hp 1–3	Hp 2–3	Hp 3–3	
1–1 × 1–1	41/41	....	....	....	....	..
1–1 × 1–2	3/4	5/4	....	....	....	..
1–1 × 1–3	64/65.5	....	67/65.5	....	....	.90
1–1 × 2–3	....	2/1.5	1/1.5	....	....	..
1–1 × 3–3	....	....	50/50	....	....	..
1–3 × 1–2	2/1.75	3/1.75	0/1.75	2/1.75	....	..
1–3 × 1–3	11/12.5	....	30/25	....	9/12.5	.35
1–3 × 2–2	....	1/2.5	....	4/2.5	....	..
1–3 × 2–3	....	0/.25	0/.25	0/.25	1/.25	..
1–3 × 3–3	....	....	20/16.5	....	13/16.5	.31
3–3 × 2–3	....	....	....	1/1	1/1	..
Total	121/124.7	11/10	168/160.5	7/5.5	24/30.25	.70

* The column for progeny phenotype Hp 2–2 is omitted because no progeny of this phenotype were expected or observed.

the hypothesis that the syntheses of haptoglobins 1, 2 and 3 are under the control of three allelic genes. These alleles are assigned the symbols Hp^1, Hp^2 and Hp^3 respectively. The hypothesis requires that an individual exhibiting only one of the haptoglobins in its phenotype must be homozygous for the allele controlling that haptoglobin (phenotype Hp 2-2 has the genotype Hp^2Hp^2); that an individual exhibiting two haptoglobins in its phenotype must be heterozygous for the alleles controlling those haptoglobins (phenotype Hp 1-3 has the genotype Hp^1Hp^3), and that no phenotypes containing all three haptoglobins should occur.

As indicated in Table 1, only those progeny phenotypes expected from the

above hypothesis were observed in each mating class. The expected frequencies of phenotypes among matings were calculated on the basis of the above hypothesis and compared with the observed frequencies. The probability values for the various x^2 tests are listed in the right-hand column of Table 1. These results support the hypothesis given above.

The frequencies of the alleles among the Yorkshire progeny (0.60 Hp^1, 0.00 Hp^2 and 0.40 Hp^3) and among the Landrace progeny (0.67 Hp^1, 0.05 Hp^2 and 0.28 Hp^3) were significantly different ($P = < 0.0003$).

SUMMARY

1. Using starch gel electrophoresis, ten haptoglobin fractions (Hp 1-Hp 10) have been observed in sera collected from Yorkshire and Landrace pigs and from the reciprocal crosses between these breeds.

2. Individuals exhibit one of six serum phenotypes with respect to haptoglobins 1, 2 and 3. Three of these phenotypes possess only one of the three haptoglobins (phenotypes Hp 1-1, Hp 2-2 and Hp 3-3), while the others possess two of the three haptoglobins (phenotypes Hp 1-2, Hp 1-3 and Hp 2-3). None of the samples analysed contained all three haptoglobins, nor were any devoid of all three haptoglobins.

3. Data are presented which support the hypothesis that the syntheses of haptoglobins 1, 2 and 3 are under control of alleles Hp^1, Hp^2 and Hp^3 respectively, and that an individual possessing only one of the haptoglobins is homozygous for the allele controlling the synthesis of that haptoglobin (phenotype Hp 2-2 has the genotype Hp^2Hp^2) and that an individual possessing two of the haptoglobins is heterozygous for the alleles controlling those haptoglobins (phenotype Hp 1-3 has the genotype Hp^1Hp^3).

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INACTIVATION AND MUTATIONS IN MYCOBACTERIUM AVIUM BY DECAY OF INCORPORATED RADIOACTIVE PHOSPHORUS

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INACTIVATION of bacteria and bacteriophages by decay of incorporated radioactive phosphorus (P^{32}) has been reported by STENT and FUERST (1955), FUERST and STENT (1956), STRAUSS, VAHARU, FRICKEY and MATHESON (1956), TESSMAN, TESSMAN and STENT (1957), MCFALL, PARDEE and STENT (1958), and KAUDEWITZ, VIELMETTER and FRIEDRICH-FREKSA (1958). These authors have suggested that the P^{32} disintegrations which occur in the bacterial deoxyribonucleic acid (DNA) may be mainly responsible for the inactivation of the cell. Working with an avirulent strain of *Mycobacterium avium*, the present author (TSUKAMURA 1960a) has stated that the mutagenic effect of radioactive isotopes is not always parallel with the radioactivity and that radioactive phosphorus, which can incorporate into the DNA, can exhibit a significant mutagenic effect, while radioactive sulfur, which cannot incorporate into the DNA, can exhibit no significant mutagenic effect. These results have indicated that the mutagenic effect is affected by the intracellular localization of the isotope. The present paper concerns relationship between the intracellular distribution of P^{32} and the inactivation and mutations induced by the presence of P^{32}. The results have revealed that inactivation and mutations in this organisms are produced rather by the decay of incorporated radioactive phosphorus (change of P^{32} into S^{32}) rather than by the beta-ray irradiation.

MATERIALS AND METHODS

Strain: Mycobacterium avium, an avirulent strain Jucho (S type), was used throughout. This strain was subcultured in modified Sauton medium at 37°C for seven days and then washed three times in physiological saline (0.9 percent NaCl solution). This culture age corresponded to the stationary growth phase and contained about 5×10^7 visible cells or about 5×10^6 viable cells per mg wet weight (TSUKAMURA 1960c). The modified Sauton medium consists of 4.0 gm of sodium glutamate, 0.5 gm of KH_2PO_4, 0.5 gm of $MgSO_4$-$7H_2O$, 0.05 gm of ferrous ammonium citrate, 2.0 gm of sodium citrate, 40 ml of glycerol, and 960 ml of distilled water. The *p*H of the medium was adjusted to 7.0 by sodium hydroxide. The medium was dispensed in 50 ml amounts into 200 ml Erlenmeyer flasks and sterilized by autoclaving at 115°C for 20 minutes.

Labeling of cells by P^{32}: Cells were homogenized by shaking with glass beads for ten minutes and were incubated in solutions containing P^{32}-orthophosphate.

Two major procedures were used for labeling of cells by P^{32}. (1) In order to increase incorporation of P^{32} into cellular fractions other than DNA and, at the same time, to decrease incorporation into the DNA, the cells were incubated at 37°C in saline containing relatively high concentrations of P^{32}-orthophosphate. (2) On the other hand, in order to increase incorporation of P^{32} into the DNA, the cells were incubated at 37°C in phosphorus-deficient medium containing relatively low concentrations of P^{32}-orthophosphate as a sole phosphorus source. The phosphorus-deficient medium consists of the modified Sauton medium from which phosphate has been omitted.

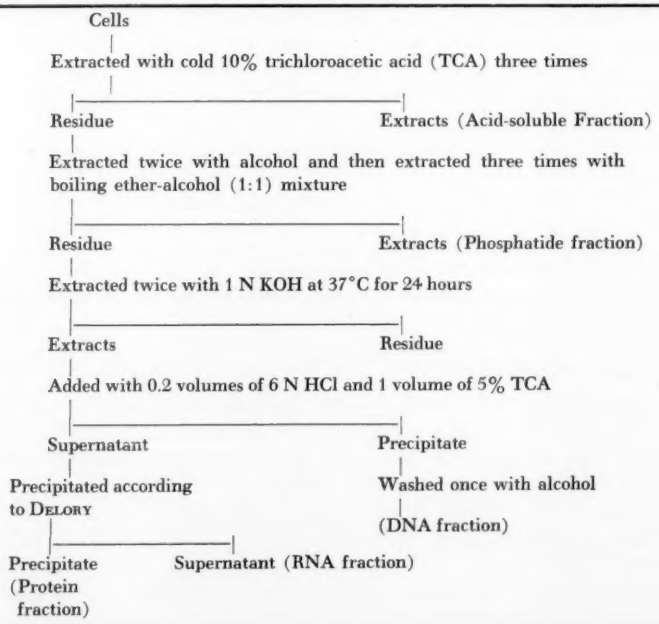
P^{32}-orthophosphate (specific activity: C.F.; radiochemical purity: 99 percent) was supplied by the Radiochemical Center, Amersham, England.

Fractionation of phosphorus compounds and determination of radioactivity: The cells labeled with P^{32} were washed repeatedly in 20 volumes of physiological saline until a 0.2 ml sample of the washing water had no significant radioactivity. Usually, five washings were satisfactory for this purpose.

One portion of the washed cells were once again washed in distilled water, weighed and fractionated according to the procedure of SCHMIDT and THANNHAUSER (1945) (Table 1). The remaining portion of the washed cell mass was stored in a refrigerator and used for experiments.

TABLE 1

Fractionation of cells according to the procedure of SCHMIDT *and* THANNHAUSER


Cells
Extracted with cold 10% trichloroacetic acid (TCA) three times
Residue
Extracts (Acid-soluble Fraction)
Extracted twice with alcohol and then extracted three times with boiling ether-alcohol (1:1) mixture
Residue
Extracts (Phosphatide fraction)
Extracted twice with 1 N KOH at 37°C for 24 hours
Extracts
Residue
Added with 0.2 volumes of 6 N HCl and 1 volume of 5% TCA
Supernatant
Precipitate
Precipitated according to DELORY
Washed once with alcohol
(DNA fraction)
Precipitate (Protein fraction)
Supernatant (RNA fraction)

Each 0.2 ml of the fractions obtained was placed in a stainless steel planchet and heated to dryness. The radioactivity was counted by a Geiger-Mueller counter (Phillips, Holland, GM 481D). The amounts of P^{32}-isotope were expressed as "counts per minute" (cpm) per mg, wet weight, of cells or per μg of DNA. The dry weight was 20±1 percent of the wet weight in the test organism. The amount of DNA was determined by the diphenylamine reaction (DISCHE 1930) in the DNA fraction. Appropriate corrections were made for natural decay and for background.

The radioactivity of whole cells also was determined prior to the fractionation. Each 0.2 ml of the appropriate cell suspension was placed in a stainless planchet and heated to dryness, and its radioactivity was measured. The radioactivity of whole cells was expressed as cpm per mg, wet weight, of cells.

Determination of the number of surviving cells and of the numbers of streptomycin-resistant mutants and isoniazid-resistant mutants in the surviving fraction: The radioactive, washed cell mass was stored at −7°C (±2°C) after washing as described above. This refrigeration of the cells considerably decreased the number of surviving (colony-developing) cells during the first 24 hours of refrigeration. Subsequently, experiments were begun taking the second day of refrigeration as the zero time. As shown later, inactivation of bacteria occurred slowly also on further refrigeration (Figure 1). Therefore, the amount of survival fraction in radioactive cells was determined by subtracting the decrease in viable number in nonradioactive cells (control, Figure 1) from the amount of the survival fraction estimated in the labeled cells.

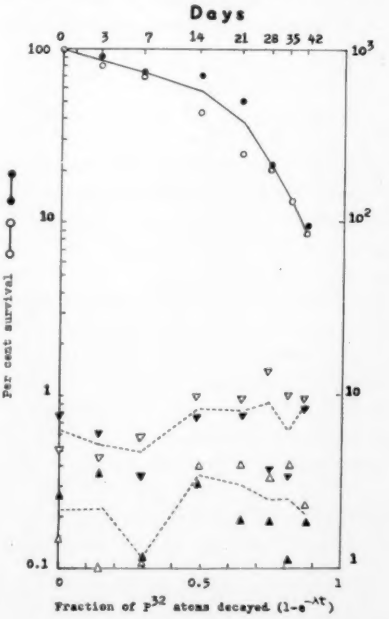


FIGURE 1.—Percent survival and mutation frequencies to isoniazid resistance and to streptomycin resistance in nonradioactive cells (control) which were stored at −7°C in a refrigerator. (Cells were washed in saline three times and then suspended in phosphorus-deficient medium (without P^{32}) at 37°C for 24 hours. The cells, thereafter, were washed in saline three times and refrigerated. Open and closed marks indicate independent determination respectively. Lines indicate averages of two experiments.

Mutations to isoniazid resistance per 10^5 surviving organisms ▽ ▼.

Mutations to streptomycin resistance per 10^5 surviving organisms △ ▲.

At appropriate time intervals, a portion of the refrigerated cell mass was homogenized by shaking with glass beads for ten minutes and was suspended in saline. The optical density of an appropriate dilution of the cell suspension was measured by a Leitz's Rouy-Photometer; the number of milligrams, wet weight, of cells per ml of suspension was determined by comparing readings with a previously prepared standard curve.

Ratios of streptomycin-resistant mutants and isoniazid-resistant mutants, arising spontaneously or induced by the presence of P^{32}, occurring in the total survival population were used as markers of the mutation frequencies. Counts were performed in the conventional manner by utilizing Sauton agar plates. Sauton agar medium consisted of modified Sauton medium added to three percent agar. One tenth ml of each of the different dilutions of cell suspension was inoculated onto each of five plates, nine cm in diameter; containing no drug, containing 1 μg of streptomycin per ml, and containing 10 μg of isoniazid per ml. Plates were incubated at 37°C for five days, and the number of colonies was counted in the usual manner. Sterile dihydrostreptomycin sulfate (manufactured by Meiji Co.) and isoniazid (manufactured by Tanabe Co.) were added to Sauton agar media immediately before use.

Amounts of the survival fraction were determined as numbers of surviving organisms per mg, wet weight, and then survival ratios were calculated taking the survival fraction at the zero time as the unit.

Figures shown in the present study were plotted without correcting decays of P^{32} occurring during the first 24 hours of refrigeration and during the incorporation process. However, errors derived from those are believed to be minor ones because of a relatively short time of those periods when compared with the total time of observation.

RESULTS

Conditions for labeling of cells by P^{32} are shown in Table 2.

Distribution of radioactivity of P^{32} between the bacterial cells and the supernatant and washing waters, ratio of uptake of P^{32} by bacterial cells, distribution of radioactivity in cellular fractions fractionated according to the procedure of SCHMIDT and THANNHAUSER, and radioactivity of the DNA fraction per μg of DNA are shown in Table 3.

P^{32} decay-survival curves and P^{32} decay-mutation curves are shown in Figures 2 to 7. Since, as has been stated, some part of the frozen cells lost their viability during the refrigeration, decay-survival curves were plotted by subtracting decrease of survival due to the refrigeration alone (Figure 1) from data of the survival fraction in P^{32}-labeled cells. On the other hand, the mutation frequencies, ratios of mutants in total surviving population, were not affected by the refrigeration (Figure 1). Therefore, data of the mutation frequencies in the labeled cells were plotted without any correction.

In the present study, experiments A and B, C and D, and E and F were performed in pairs, respectively.

TABLE 2

Labeling condition of bacterial cells

Experiment	Condition of labeling of cells with P^{32}
A	Cells (113 mg/ml) were incubated in saline containing 1.5 microcuries P^{32}-orthophosphate/ml for three hours
B	Cells (134 mg/ml) were incubated in phosphorus-deficient medium containing 0.3 microcuries P^{32}-orthophosphate/ml for three days (before labeling, the cells were preincubated in phosphorus-deficient medium for one day to exhaust their phosphorus deposit)*
C	Cells (94 mg/ml) were incubated in saline containing 3.0 microcuries P^{32}-orthophosphate/ml for three hours
D	Cells (132 mg/ml) were incubated in phosphorus-deficient medium containing 1.0 microcuries P^{32}-orthophosphate/ml for 24 hours*
E	Cells (80 mg/ml) were incubated in saline containing 3.0 microcuries P^{32}-orthophosphate/ml for 24 hours
F	Cells (88 mg/ml) were incubated in phosphorus-deficient medium containing 0.5 microcuries P^{32}-orthophosphate/ml for 24 hours (before labeling, the cells were preincubated in phosphorus-deficient medium for three days to exhaust phosphorus deposit)*

* P^{32}-orthophosphate was added as a sole phosphorus source.

Ratio of uptake of P^{32} by bacterial cells, radioactivity of whole bacterial cells and distribution of radioactivity in cellular fractions: These varied according to the labeling condition (Tables 2 and 3). As had been expected, incubation of cells in phosphorus-deficient media containing P^{32} as a sole phosphorus source represented an increased ratio of uptake of P^{32} by cells and an increased amount of P^{32} incorporated into the DNA fraction, accompanying frequently a relative decrease of radioactivity in the acid-soluble fraction (Experiments B, D and F). On the other hand, generally reverse relations were found in cells incubated in saline containing P^{32} (Experiments A, C and E).

2. Decay-survival curves and decay-mutation curves: Percent survival and mutation frequencies were expressed as a function of P^{32} decay. These are shown in Figures 2 to 7 and partially summarized in Table 4.

(1) Decay-survival curves. Different types of survival curves were found (Figures 2 to 7). It was remarkable that inactivation of cells (loss of ability to develop colonies) was much more significantly affected by the amount of P^{32} found in the DNA fraction than done by the amount of total radioactivity of cells which must parallel the amount of beta-ray irradiation.

As shown in figures, inactivation occurred most rapidly in experiment B. The order in respect to the rapidity of inactivation was as follows: Experiments B, F, D, C, E, A. On the other side, the amount of total radioactivity was the highest in experiment E. The order in respect to the amount of total radioactivity, total amount of P^{32} contained in cells, was as follows: E, C, F, D, B, A. This order is apparently incompatible with the former order concerning inactivation.

The amount of radioactivity (P^{32}) in the DNA fraction was the highest in experiment B, and the order in respect to the amount is as follows: B, E, F, D, C,

TABLE 3

Distribution of radioactivity in culture, uptake ratio of P^{32} by bacterial cells, radioactivity of whole bacterial cells, and distribution of radioactivity in cellular fractions

	Experiment A	Experiment B	Experiment C	Experiment D	Experiment E	Experiment F
Amount of bacterial cells used:	3,390 mg	4,000 mg	3,780 mg	5,300 mg	3,200 mg	4,420 mg
Distribution of radioactivity in culture:						
(1) Bacterial cells	157,000 cpm	884,000 cpm	1,150,000 cpm	1,324,000 cpm	6,720,000 cpm	1,569,000 cpm
(2) Supernatant and washing waters	2,990,000 cpm	133,000 cpm	10,568,000 cpm	4,140,000 cpm	4,075,000 cpm	399,000 cpm
(3) Total	3,147,000 cpm	1,017,000 cpm	11,718,000 cpm	5,464,000 cpm	10,795,000 cpm	1,968,000 cpm
Uptake ratio of P^{32} by bacterial cells:	5.0 percent	87.0 percent	9.8 percent	24.2 percent	62.2 percent	80.0 percent
Radioactivity of whole cells:	46.3 cpm/mg	221. cpm/mg	314. cpm/mg	250. cpm/mg	2,096. cpm/mg	355. cpm/mg
Distribution of radioactivity in cellular fractions: *	cpm/mg	cpm/mg	cpm/mg	cpm/mg	cpm/mg	cpm/mg
(1) Acid-soluble fraction	18.1 (38.8%)	43.2 (26.9%)	80.0 (28.2%)	48.6 (22.7%)	410. (27.0%)	63.5 (17.8%)
(2) Phosphatide fraction	3.15 (6.7%)	2.64(1.6%)	4.74(1.7%)	13.5 (6.3%)	81.0 (5.3%)	11.7 (3.3%)
(3) DNA fraction	0.086(0.18%)	14.4 (8.9%)	3.89(1.4%)	6.3 (2.9%)	13.9 (0.91%)	10.2 (2.9%)
(4) RNA fraction	21.3 (45.7%)	85.0 (52.6%)	173. (61.0%)	111.3 (51.9%)	825. (54.2%)	233. (65.5%)
(5) Protein fraction	4.00 (8.6%)	16.2 (10.0%)	22.5 (7.9%)	34.6 (16.2%)	190. (12.6%)	37.6 (10.6%)
(6) Total	46.7 (100%)	161.4 (100%)	284.1 (100%)	214.3 (100%)	1,520. (100%)	356.0 (100%)
Radioactivity of the DNA fraction per one μg of DNA:	0.029 cpm/ μg DNA	4.76 cpm/ μg DNA	1.30 cpm/ μg DNA	2.10 cpm/ μg DNA	4.62 cpm/ μg DNA	3.21 cpm/ μg DNA

mg = mg in wet weight.

* Fractionated according to the procedure of SCHMIDT and THANNHAUSER.

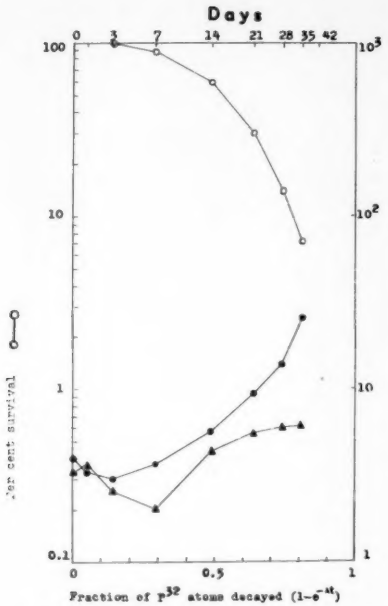


FIGURE 2.—Decay-survival curve and decay-mutation curves in experiment A (see Tables 2 and 3).

Mutations to isoniazid resistance per 10^5 surviving organisms ●—●.

Mutations to streptomycin resistance per 10^5 surviving organisms ▲—▲.

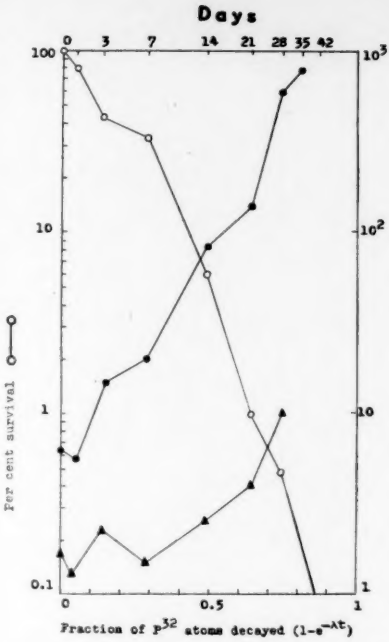


FIGURE 3.—Decay-survival curve and decay-mutation curves in experiment B (see Tables 2 and 3).

Mutations to isoniazid resistance per 10^5 surviving organisms ●—●.

Mutations to streptomycin resistance per 10^5 surviving organisms ▲—▲.

TABLE 4

Correlations among total radioactivity of cells, radioactivity of DNA fraction, inactivation of cells, and induced mutation in Mycobacterium avium *(Jucho)*

Exp.*	Radioactivity of whole cells in cpm/mg, wet weight	Radioactivity of DNA fraction in cpm/mg, wet weight	DNA cpm Total cpm†	Percent survival (after 14 days)	Percent survival (after 28 days)	Increase of mutation to isoniazid resistance
A	46.3 cpm/mg	0.086 cpm/mg	0.18%	59.0%	14.0%	1.4 times
B	221.	14.4	8.9	5.6	0.5	12.6
C	314.	3.89	1.4	21.0	4.7	8.8
D	250.	6.30	2.9	10.0	2.8	13.0
E	2,096.	13.9‡	0.91	6.4	3.4	10.7
F	255.	10.2	2.9	6.9	0.6	10.8

* Experiments A and B, experiments C and D, and experiments E and F were performed as a pair experiments, respectively.

† Percentage of radioactivity of the DNA fraction per total radioactivity.

‡ This amount was suggested to be derived from contamination of P^{32} (see text).

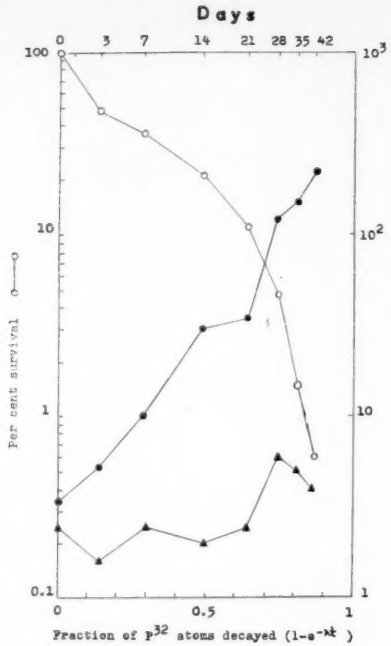


FIGURE 4.—Decay-survival curve and decay-mutation curves in experiment C (see Tables 2 and 3).

Mutations to isoniazid resistance per 10^5 surviving organisms ●—●.

Mutations to streptomycin resistance per 10^5 surviving organisms ▲—▲.

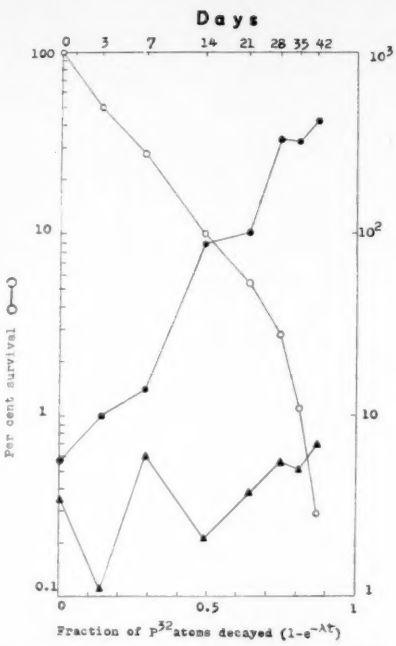


FIGURE 5.—Decay-survival curve and decay-mutation curves in experiment D (see Tables 2 and 3).

Mutations to isoniazid resistance per 10^5 surviving organisms ●—●.

Mutations to streptomycin resistance per 10^5 surviving organisms ▲—▲.

A. This order is apparently compatible with the order in respect to the rapidity of inactivation of cells, although experiment E only was an exceptional case.

In experiments E and F (Tables 2 and 3 and Figures 6 and 7), which were performed as a pair at the same time, the cells in experiment E showed a somewhat higher P^{32} amount in the DNA fraction than those in experiment F. Inactivation of cells occurred, in contrary, much more rapidly in the cells of experiment F. This phenomenon would be interpreted as follows. In these experiments, the DNA fraction contained usually a relatively low amount of radioactivity. Subsequently, it appears quite possible that the DNA fraction is contaminated by the other fractions containing relatively high amounts of P^{32}. Since the cells of experiment E contained the highest amount of radioactivity as whole cells, radioactivity in the DNA fraction in experiment E is thought to be increased by such contamination. Thus, the DNA fraction of experiment E might represent a higher amount of P^{32} than is actually present.

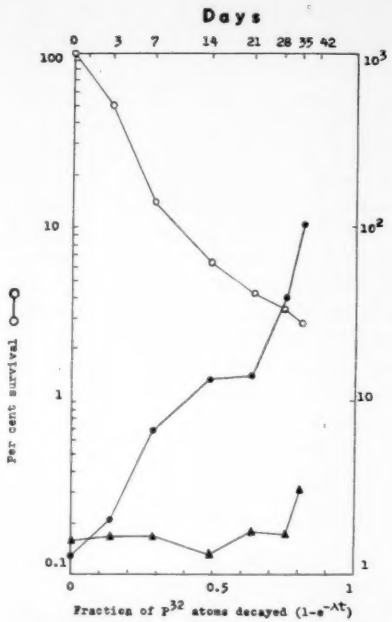


FIGURE 6.—Decay-survival curve and decay-mutation curves in experiment E (see Tables 2 and 3).

Mutations to isoniazid resistance per 10^5 surviving organisms ●—●.

Mutations to streptomycin resistance per 10^5 surviving organisms ▲—▲.

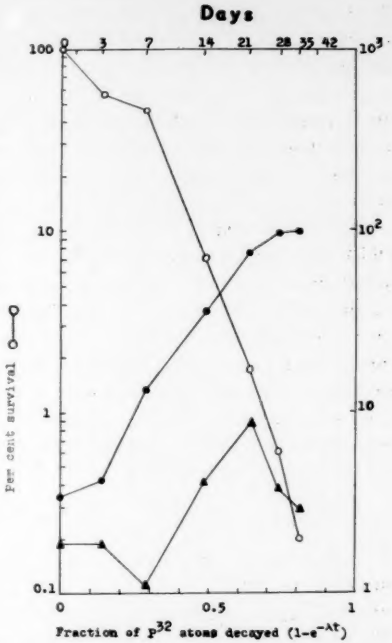


FIGURE 7.—Decay-survival curve and decay-mutation curves in experiment F (see Tables 2 and 3).

Mutations to isoniazid resistance per 10^5 surviving organisms ●—●.

Mutations to streptomycin resistance per 10^5 surviving organisms ▲—▲.

It is apparent that the amount of total radioactivity, which parallels the amount of beta-ray irradiation, has no close correlation with the incidence of inactivation. The ratio of uptake of P^{32} may have any positive correlation with the inactivation, although a definite conclusion was not made from the present data. The amounts of radioactivity in other cellular fraction did not appear to have any close correlation with the occurrence of inactivation.

In addition, the data on decay-survival curves appear to indicate a tendency that the higher the amount of P^{32} in the DNA fraction, the more exponential becomes the decay-survival curves (Figures 2 to 7 and Table 3 and 4). This finding appears to indicate an important role of P^{32} decay in the DNA fraction for the induction of cell inactivation. For example, the decay-survival curves in experiments B, E and F (Figures 3, 5 and 7), of which the DNA fractions contained relatively high amounts of P^{32}, resemble the exponential curve. On the other side, there appears to be also the tendency that the lower the amount of P^{32} in the

DNA fraction, the curve resembles a multihit curve. The decay-survival curves of experiments A and C (Figures 2 and 4) more or less resemble the multihit curves.

(2) Decay-mutation curves. In the decay-mutation curves (Figures 2 to 7), the most striking fact is a discrepancy between mutations to isoniazid resistance and to streptomycin resistance. The mutation frequency to isoniazid resistance was rapidly increased as the P^{32} decay progressed, while the mutation frequency to streptomycin resistance was not increased or only slightly increased.

There appeared to exist the tendency that the higher the amount of P^{32} in the DNA fraction, the more rapid becomes the decay-mutation (to isoniazid-resistance) curve, and, in most cases, the decay-mutation curve tended to be as if exponential to the decay of P^{32}. However, further experiments are needed to determine whether or not this correlation indeed exists since this correlation was not as clear as that found in the decay-inactivation curves. Determination of the mutation frequency involved a larger number of errors than did the determination of the survival, for the former contained errors involved in both determinations of the number of surviving cells and of the number of mutants.

DISCUSSION

Experimental conditions: Experiments in the present study were performed under the conditions excluding any selective process. As seen in control experiment (Figure 1), the refrigeration does not increase the mutation frequency. Therefore, the procedure of refrigeration is not selective for the marker mutants. Both procedures of incubation of cells in physiological saline as well as in phosphorus-deficient medium did not show any significant change of turbidity of cells. Therefore, these are believed to be unselective. It is also believed that any change of intracellular distribution of P^{32} did not occur after the determination on distribution of P^{32}, since the cells were stored at a freezing state during the progress of P^{32} decay.

In addition, it appears better to describe some additional properties of the test organism. The strain Jucho of *Mycobacterium avium* is inactivated by ultraviolet irradiation showing survival curves of a single-hit type, like other mycobacteria (TSUKAMURA 1960b). Furthermore, this strain is a member of the group showing the lowest DNA content per mg, wet or dry weight, in the genus Mycobacterium (unpublished data).

Mode of inactivation of cells: As stated above, it is remarkable that inactivation is affected by the amount of radioactivity in the DNA fraction rather than by the amount of total radioactivity that parallels the amount of beta-ray irradiation. If inactivation were caused by beta-ray irradiation, the rapidity of inactivation would parallel the amount of total radioactivity. This is not the case, as described above. Inactivation occurs showing a close correlation with the amount of P^{32} in the DNA fraction. The decay-survival curves tended to be exponential, as the amount of P^{32} in the DNA fraction increased. These findings strongly suggest that inactivation of cells is caused by decay of P^{32}—change of P^{32} into

S^{32}—in the DNA fraction. This change must cause a change of the DNA structure. However, it is unlikely that the decay of P^{32} in the DNA fraction is the sole mechanism responsible for cell inactivation although it is believed to be the most important factor.

As shown in experiments A and C, especially in the former (Figure 2), cells having a slight labeling of the DNA fraction by P^{32} represented decay-survival curves of a multihit type. The existence of decay-survival curves of the multihit type indicate that inactivation may occur by decay of incorporated P^{32} in multiple loci. These multiloci seem to be outside of the DNA on the basis of the following points: (1) The existence of decay-survival curves of the single-hit type, or resembling it, in cells showing heavy labeling of the DNA fraction would suggest the existence of only one nucleus according to the target theory. (2) It is not practical to suppose the existence of such an excess of nuclei as considered from the shape of the decay-survival curve in experiment A (Figure 2). Subsequently, these multiloci appear to be in the cytoplasm, and it is possible that they may be ribosomes.

There are two conflicting claims as to the number of nuclei of mycobacteria by cytochemical observations. KNAYSI (1952, 1957, 1959) and KNAYSI, HILLIER and FABRICANT (1950) claimed that mycobacteria possess several nuclei. On the other hand, MUDD and others (MUDD, WINTERSCHEID, DELAMATER and HENDERSON 1951; WINTERSCHEID and MUDD 1953; TODA, KOIKE, HIRAKI and TAKEYA 1957) claimed that mycobacteria have only one (or two) nucleus. The results of the present study would support the one-nucleus theory.

The test organism contained about 5×10^7 cells per mg (wet weight). However, the number of viable cells per mg was determined to be about 5×10^6 per mg. wet weight, under the culture age used for experiments. If one supposed that only viable cells could incorporate P^{32} into their DNA, it would be possible that, under the conditions shown in the experiment B (Figure 3), only a few disintegrations of DNA-P^{32} atoms per cell may be sufficient to cause cell inactivation, although this supposition remains yet impractical because of considerable experimental errors.

Mutations induced by P^{32} decay: As stated above, there is no close correlation between the induced mutation and the amount of total radioactivity of cells, which parallels the amount of beta-ray irradiation. Consequently the induced mutation is not thought to be mainly derived from the beta-ray irradiation. It appears noteworthy that the decay-mutation curves were roughly exponential to the P^{32} decay (Figures 3 to 7). It appears also that the induced mutation frequency may be roughly in accordance with the amount of P^{32} incorporated into the DNA fraction (Table 4), although this could not be accurately demonstrated, possibly, because of the inaccuracy of determinations of the mutation frequency. In view of the findings, it appears apparent that the induced mutation is in some manner derived from the P^{32} decay.

Mutations (to isoniazid resistance) could occur even under the conditions in which P^{32} was scarcely contained in the DNA fraction (Figure 2). These mutations appear to result from the following possibilities: (1) mutations by decay

of P^{32} atoms in the DNA; (2) mutations derived from abnormal protein synthesis caused by decay of P^{32} in the cytoplasm; (3) mutations by beta-ray irradiation. The third possibility appears to be unimportant in the test organism, for the beta-ray irradiation has had no significant mutagenic effect on the organism (TSUKAMURA 1960a). The second possibility may be present, considering the data shown by WITKIN (1956), HAAS and DOUDNEY (1957) and DOUDNEY and HAAS (1958, 1959). Mutations from such origin would be possible in respect to isoniazid resistance but would be impossible in respect to streptomycin resistance (streptomycin inhibits protein synthesis).

The existence of discrepancy between mutations to isoniazid resistance and to streptomycin resistance was a remarkable phenomenon. The same phenomenon had been observed in the induced mutations by ultraviolet irradiation (TSUKAMURA 1960b). At that time, the phenomenon was interpreted by the presence of some phenomic lag for phenotypic expression of mutation to streptomycin resistance, while there is no phenomic lag for expression of isoniazid resistance. Since experiments of the ultraviolet study were performed concerning the zero point mutation, it was suggested that low mutation frequency to streptomycin resistance is derived from delay of phenotypic expression. In experiments of the present study, the cells were inoculated immediately on media containing isoniazid or streptomycin. Therefore, the mutation frequencies observed concern the zero point mutation. The discrepancy may be derived from the absence or presence of phenomic lag in mutations to isoniazid resistance and to streptomycin resistance.

Previously, the author (TSUKAMURA 1960a) found that cells of this organism growing actively in Sauton medium containing 0.5 microcuries of P^{32}-phosphate per ml represented a marked increase of the both mutation frequencies, unlike the results of the present study. That finding is interpreted by assuming that, in growing cultures, induced streptomycin-resistant mutants could accomplish their phenotypic expression.

SUMMARY

Inactivation and mutation occurred in Mycobacterium Jucho by decay of incorporated radioactive phosphorus (P^{32}) under the conditions in which cells labeled with P^{32} were stored in freezing state during the progress of P^{32} decay, and the existence of any selective process of marker mutants could be excluded.

Relationships between the inactivation and mutations and the intracellular distribution of P^{32} were studied. Fractionation of radioactive cells was performed according to the procedure of SCHMIDT and THANNHAUSER.

Inactivation of cells was much more markedly affected by the amount of P^{32} in the DNA fraction than by the amount of total radioactivity of cells that parallels the amount of beta-ray irradiation. Inactivation curves, survival curves plotted as a function of the amount of P^{32} decay tended to be exponential, if the DNA fraction was labeled heavily by P^{32}. It is strongly suggested, thus, that beta-ray irradiation is not responsible for inactivation of bacterial cells and decay of

P^{32} in the DNA fraction, but that change of P^{32} into S^{32} in DNA structure is mainly responsible for it.

If cells were labeled by P^{32} representing a relatively low content of P^{32} in the DNA fraction, inactivation of cells occurred showing decay-survival curves of a multihit type. Thus, it is also suggested that inactivation of cells may occur also by decay of P^{32} in multiloci outside of the DNA.

Mutation frequency to isoniazid resistance was rapidly increased with progress of P^{32} decay, while mutation frequency to streptomycin resistance was not increased or only slightly increased. Thus, a marked discrepancy between these two mutations was observed. Mechanism of this discrepancy was discussed. The incidence of mutation (to isoniazid resistance) did not parallel the amount of total radioactivity of cells that corresponded to the amount of beta-ray irradiation. The induced mutation was increased roughly in accordance with the progress of P^{32} decay. It is conceivable that the induced mutation also occurs by decay of incorporated P^{32}.

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THE GENETICS AND BIOCHEMISTRY OF RED FAT CELLS IN DROSOPHILA MELANOGASTER[1]

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IN the strain of Drosophila known as "red fat cells" conspicuous pigmented cells are visible in the head and thorax of the flies (JONES and LEWIS 1957). Normally colorless fat cells acquire a pigment that is related to, or perhaps identical with, the brown ommochrome derived from tryptophan.

This report concerns some properties of the red fat cells (RFC) strain, two of which have been briefly reported (GRELL 1958). The larvae, pupae and adults of the RFC strain contain much more of the amino acid, lysine, than wild-type animals. The RFC phenotype is the result of an interaction between two mutants, lysine (*lys*) and red cells (*rc*). Each of these mutant loci seems to have the tendency to produce pigmented fat cells in the absence of the other, but this tendency is expressed only under special conditions.

BIOCHEMICAL ASPECTS

Accumulation of lysine: That Drosophila of the RFC strain contain more lysine than wild type was demonstrated largely by paper chromatography. The chromatographic techniques were essentially those first employed on Drosophila by HADORN and MITCHELL (1951). All chromatograms of larvae, pupae and adults of the RFC strain showed that one ninhydrin-positive spot was much larger and darker than the corresponding spot produced from wild-type Canton-S animals. Several solvent systems were used and in all cases the Rf values of the accumulated amino acid were equivalent to that of lysine.

Two dimensional patterns resulting from paper electrophoresis in one dimension and chromatography with butanol-1:acetic acid:water (11:3:4) in the second dimension clearly show the lysine accumulation in RFC animals (Figure 1).

A further indication that the accumulated amino acid is lysine was a test of its ability to act as a growth factor for a lysineless mutant (37811a) of Neurospora. The accumulation isolated from chromatograms of RFC animals contained more growth factor than material isolated from the same region of chromatograms of Canton-S animals.

[1] Parts of this material were submitted to the California Institute of Technology in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

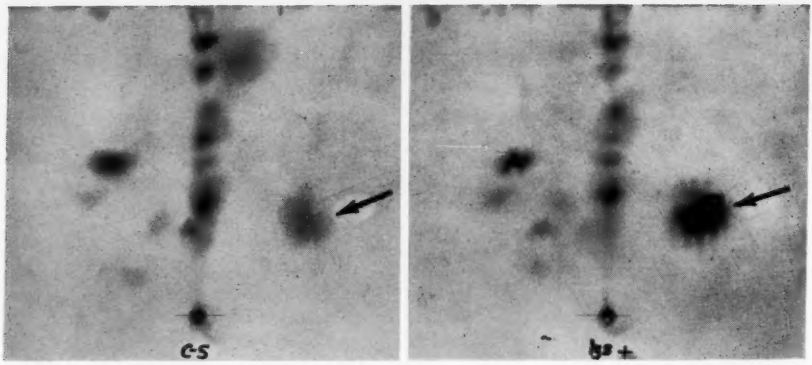

FIGURE 1.—Canton-S and the *lys* mutant compared by paper electrophoresis in horizontal direction and chromatography in butanol-acetic acid-water in the vertical direction. Paper sprayed with ninhydrin solution. Arrows point to the lysine spots.

Injections of L-lysine-C^{14}: In order to learn the cause of the abnormally large amounts of lysine in the RFC strain, C^{14}-labeled lysine was injected into RFC and wild-type adult flies. The amount of radioactivity was measured in respired CO_2 and other fractions of the flies.

Fractions of carbon dioxide were collected by precipitation as $BaCO_3$ for eight hours after injection of lysine. The flies were then ground with sand and extracted with solvents. In Experiment I the animals were extracted only with boiling water, but in Experiment II they were first extracted with ether and then with boiling water. The tube which had contained the injected flies for eight hours was rinsed with hot water to recover the excretory products. The residue was the material that was insoluble in ether and water or was precipitated by exposure to boiling water, as in the case of some proteins. Table 1 lists the results of Experiments I and II.

The distribution of radioactivity recovered from Canton-S flies was quite different from the distribution from the RFC strain. Canton-S animals converted on the average 37.9 percent of the recovered radioactivity into CO_2, but RFC animals converted only 3.0 percent into CO_2. In other words, Canton-S flies converted 12.6 times more lysine into CO_2 in an eight-hour period than did RFC flies.

Most of the radioactive lysine injected into RFC flies was recovered in the water soluble fraction. Chromatography of this material indicated that most of it was unaltered lysine.

The amount of radioactivity in the ether soluble fractions is consistent with the results of the CO_2 tests. If in the Canton-S strain more lysine were degraded to CO_2 than in mutant flies, then more carbon atoms from lysine also would be expected in fats and other ether-soluble materials. In confirmation of this expectation, Canton-S flies had about five times more radioactivity in the ether soluble fraction than did the RFC flies (Table 1).

The greater amount of radioactivity in the excretion products of the RFC flies

TABLE 1

Results of experiments in which 25 Canton-S and 25 RFC males were injected with randomly labeled 0.1 M L-lysine-C^{14}

Fraction	Experiment I Time (min.)	Experiment I Counts per minute Canton	Experiment I Counts per minute RFC	Experiment II Time (min.)	Experiment II Counts per minute Canton	Experiment II Counts per minute RFC
CO_2	0–75	680	51	0–120	1800	120
CO_2	75–170	460	52	120–270	2200	230
CO_2	170–325	1400	150	270–480	1500	240
CO_2	325–480	1500	23	....	....	....
Total CO_2	0–480	4040	276	0–480	5500	590
Percent	....	36	2	....	40	4
Ether soluble	480	....	....	480	1300	330
Percent	....	....	....	....	10	2
Water soluble	480	5300	15000	480	3200	8330
Percent	....	47	80	....	23	61
Residue	480	1900	3400	480	2600	1700
Percent	....	17	18	....	19	13
Excretion	480	....	....	480	1100	2670
Percent	....	....	....	....	8	20
Total	....	11240	18676	....	13700	13620

may reflect a real difference although this category is subject to error. Since free lysine remains at a high concentration in RFC animals, more of it might be excreted than in normal animals where a large proportion is degraded to carbon dioxide.

The radioactivity in the residue is probably an index of the lysine incorporated into protein. In Canton-S animals 18.2 percent, and in RFC animals 15.6 percent, of the recovered counts were in the residue. This probably indicates no real difference. The amount of radioactive lysine incorporated into protein might be expected to be slightly lower in RFC flies because at the time of injection there is a larger pool of unlabeled lysine in RFC flies and this unlabeled lysine would tend to dilute the radioactive material.

GENETICS OF RED FAT CELLS

The recessive factor responsible for the RFC phenotype has been located by LEWIS (1950) between Sternopleural (*Sp*) and Jammed (*J*) at approximately 26 on the second chromosome. In experiments to locate the factor more accurately, the two distinct abnormal phenotypes, red fat cells and accumulation of lysine, were classified independently.

Females of the constitution, *Sp* RFC *J*/+ + +, were produced and crossed to Canton-S males. In the first generation the offspring could not be scored for the recessive RFC factor or lysine; however they were scored for crossing over between *Sp* and *J*. Males possessing a crossover chromosome were mated to yellow forked double X; RFC virgin females (*y f*:=; RFC) and the *Sp* or *J* progeny were scored for red fat cells. In addition, a sample of flies bearing each crossover was

tested for lysine accumulation by chromatography. In another cross *Sp* RFC *J*/*d* females (*d* = dachs) were crossed to Canton-S males and the crossover chromosomes tested as above.

In the first generation the progeny was distributed as follows:

Parental:	Recombinant:
2441 *Sp J*	259 *Sp* +
2532 + +	263 + *J*

There is 9.5 percent recombination between *Sp* and *J*. Results of progeny tests of male recombinants are as follows:

109 + + + *J*	52 *Sp* lysine RFC +
10 + lysine RFC *J*	54 *Sp* lysine + +
0 + lysine + *J*	11 *Sp* + + +

The data shows that 54 of the crossovers accumulated lysine but did not have the RFC phenotype. On the other hand, all flies with the RFC phenotype accumulated lysine. The accumulation of lysine appears to be due to a single factor and the RFC phenotype is produced by an interaction between the factor for lysine accumulation and some other factor. The factor for lysine accumulation is called lysine (*lys*) and the other factor, red cells (*rc*).

From females heterozygous for dachs, 173 crossovers were scored for lysine and red fat cells. The results are given in Table 2.

TABLE 2

Genotypes of crossovers from $\frac{\text{Sp lys + rc J}}{\text{+ + d + +}}$ *females*

Region 1	Region 2	Region 3	Region 4
6 *Sp* + *d* + +	16 *Sp lys d* + +	26 *Sp lys* + + +	37 *Sp lys* + *rc* +
8 + *lys* + *J*	21 + + + *rc J*	+ + *d rc J**	+ + *d* + *J**

* 59 total in regions 3 and 4.

Combining all of the data the following map may be constructed: *Sp*—.85—*lys*—1.7—*d*—2.7—*rc*—4.2—*J*. Using the standard map location of *Sp* as 22.0, *lys* is located at 22.85 and *rc* at 27.25. It should be pointed out that there is a discrepancy between the standard distance between *Sp* and *J* (19 units) given in BRIDGES and BREHME (1944) and the one (10.5 units) that was obtained in these experiments.

The distance between *Sp* and *J* was measured in two separate experiments with females also containing *lys* and *rc* and found to be 10.5 and 9.5 percent. To test the possibility that *lys*, *rc* or another factor in that region was reducing crossing over, the region was measured in flies without *lys* or *rc*. The results of the cross of *Sp* +/+ J^{34e} females and + + males are as follows:

Parental:	Crossover:
1167 *Sp* +	135 *Sp J*
1083 + J^{34e}	155 + +

The crossing over amounts to 11.4 percent. Thus using *Sp* and *J* from different sources, the crossing over is always less than the standard distance. Combining the data from all of the crosses gives a crossover value of 10.2 percent from a total of 12,048 flies.

OTHER ALLELES AT THE *rc* AND *lys* LOCI

Red cells-2: Drosophila from the singed-4 stock kept in the California Institute of Technology collection were discovered by R. F. GRELL also to possess red fat cells. The pigment is located in the same cells as in *lys rc* and resembles that phenotype. There are important differences between the red cells-2 and *lys rc* phenotypes. At 17°C pigmented cells are present in rc^2 flies, but if the animals are cultured at 25°C on a usual Drosophila medium no red cells can be observed. At 25°C red cells may be detected if the rc^2 larvae have been starved. The enhancement of the RFC phenotype by starvation will be discussed later.

No biochemical difference (except the pigmentation of the fat cells) has been observed between rc^2 and wild type. There is no accumulation of lysine. The red cells phenotype in the case of rc^2 appears to be dependent upon a single gene.

By several genetic criteria, rc^2 is allelic to *rc*. Like *rc*, in crossover tests rc^2 localized to a position between *Sp* and *J*. The double homozygote, *lys* rc^2, resembles *lys rc* in that there are numerous red fat cells present at 25°C in flies cultured on a regular medium. The combination, *lys rc/lys* rc^2, has abundant red fat cells and no recombination occurs to give *lys* + from *lys rc/lys* rc^2 females.

Lysine-2: An allele of *lys* was induced by X-rays. Males carrying rc^2 on their second chromosomes were irradiated with 4000r and mated to *y; dp lys rc pr* (yellow; dumpy, lysine, red cells, purple) virgin females. From among 35,000 progeny of this cross one male with a RFC phenotype was found. He was mated to *Sp lys pr/Cy, Ins05,* dp^{tz1} *pr* cn^2 (OSTER 1956). The *Cy* dp^+ pr^+ progeny were selected to make a balanced stock which was heterozygous for the radiation-induced mutation and the balancer, *Cy, Ins05*.

A sample of the *Sp* pr^+ (*lys*^{2} rc^2/*Sp lys pr*) progeny were chromatogrammed and found to have an accumulation of lysine similar to homozygous *lys*. This constitutes an allele test, and the X-ray induced allele has been designated lysine-2 (*lys*^{2}). In addition, *lys*^{2} rc^2/*lys* + flies show some faintly pigmented fat cells. The production of red cells was unexpected since *lys* rc^2/*lys* + very rarely produces a red cell. (Under special starvation conditions, discussed later, it has been found that either homozygous rc^2 or *lys* is capable of showing a RFC phenotype in the absence of the other mutant.)

Homozygous *lys*^{2} rc^2 is poorly viable and the flies have heavy textured, slightly drooping wings. They have a RFC phenotype, but it is less extreme than *lys*^{2} rc^2/*lys rc* or *lys rc* homozygous. Attempts to separate *lys*^{2} from rc^2 were unsuccessful because *lys*^{2} is associated with a chromosomal rearrangement. There is an inversion from salivary chromosome map sections (BRIDGES 1935) 22F to 40 and a 2–3 translocation inside of the inversion with breaks at 33B in the second chromosome and 64A in the left arm of the third chromosome. The new order of

the two chromosomes is: 20 to 22F/40 to 33B/64A to 100, and 61 to 64/33B to 22F/40 to 60. The rearrangement is designated: T(2;3)lysine-2.

EFFECT OF LARVAL STARVATION ON THE RED CELL PHENOTYPE

RIZKI (1960) reported that the RFC phenotype in a combination red cells-tumor strain is expressed only if the animals are starved as larvae. It seemed desirable to determine if starvation also enhances *lys rc*, *lys* +, + *rc* and various heterozygous combinations. Experiments were performed in which larvae at the age of about 72 hours were removed from the regular Drosophila medium, washed several times in distilled water, and placed in quarter-pint milk bottles containing one inch of five percent glucose in two percent agar. The larvae worked into the agar and most of them began pupation after two days. Late pupae and young adults were examined for the presence and extent of red cells.

It was found that the homozygous *lys-rc* which had an RFC phenotype under normal culture conditions is enhanced by the starvation treatment, and some genotypes that never, or rarely, have red cells may then have some if they develop from starved larvae. The enhancement of *lys rc* by starvation was unexpected since addition of extra yeast to culture media also enhances the intensity of the phenotype (JONES and LEWIS 1957). A summary of the results is presented in Table 3. Two cases should be given special attention: (1) *lys* +/*lys* + flies

TABLE 3

Phenotypes of combinations of lys, lys^2, rc *and* rc^2

	Genotype	Phenotype	Phenotype after larval starvation
1.	*lys rc/lys rc*	lysine and RFC	lysine and RFC
2.	*lys rc/+ +*	wild type	wild type
3.	*lys rc/+ rc*	wild type	wild type
4.	*lys rc/lys +*	lysine	lysine and RFC
5.	*+ rc/+ rc*	wild type	wild type
6.	*lys +/lys +*	lysine	lysine and RFC
7.	*+ rc^2/+ rc^2*	wild type at 25°C, RFC at 17°C	RFC
8.	*+ rc^2/+ +*	wild type	wild type
9.	*lys rc^2/+ rc^2*	wild type at 25°C, RFC at 17°C	RFC
10.	*lys rc/+ rc^2*	wild type	RFC
11.	*lys rc^2/lys rc^2*	lysine and RFC	lysine and RFC
12.	*lys rc^2/lys rc*	lysine and RFC	lysine and RFC
13.	*+ rc^2/+ rc*	wild type	RFC
14.	*lys^2 rc^2/lys +*	lysine and RFC	lysine and RFC
15.	*lys^2 rc^2/lys^2 rc^2*	lysine and RFC	lysine and RFC

have red cells after starvation and (2) + *rc^2*/+*rc^2* also have red cells at 25°C if they have been starved as larvae. E. B. LEWIS (personal communication) has found that *cho* (chocolate) also enhances the red fat cell phenotype and the combination, *cho*; *lys* +, produces red fat cells.

DISCUSSION

Lysine is an essential amino acid. It must be supplied in the diet for Drosophila growth and development (HINTON, NOYES and ELLIS 1951). Drosophila which accumulate lysine as the result of being homozygous for *lys* must acquire the lysine from an external source. Drosophila grown on a regular medium (brewer's yeast, corn meal, sucrose, glucose and agar) probably ingest more lysine than is required for synthesis of proteins. In normal flies the excess lysine is broken down and metabolized.

It is believed that the effect of *lys* is, in principle, similar to some other mutations of Drosophila. GREEN (1949) showed that nonprotein tryptophan is accumulated in the vermilion mutants of both *D. melanogaster* and *D. virilis*. KIKKAWA (1941) showed that kynurenine is probably accumulated in cinnabar flies. The two mutations block different steps in the formation of brown eye pigment from tryptophan. Vermilion prevents the formation of kynurenine from tryptophan and cinnabar prevents the formation of 3-hydroxykynurenine from kynurenine.

The xanthine dehydrogenase-deficient mutants, rosy and maroon-like, also demonstrate this principle in Drosophila. The substrates of the missing enzyme, 2-amino-4-hydroxypteridine and hypoxanthine, tend to accumulate (FORREST, GLASSMAN and MITCHELL 1956; HADORN and SCHWINCK 1956; MITCHELL, GLASSMAN and HADORN 1959).

In the case of lysine accumulation, it is postulated that the gene lys^+, is necessary for rapid degradation of lysine. Since lysine itself accumulates, one might expect that it is the first step in the degradation path that is affected in flies with the *lys* mutation. ROTHSTEIN and MILLER (1954) proposed that this first step is an oxidative deamination of the alpha carbon of lysine.

There is no known biochemical reason for the relationship between lysine and the pigmentation of the fat cell. Tryptophan is the precursor of the pigment and the injection of radioactive lysine into larvae does not lead to the incorporation of radioactivity into the brown eye pigment. On the other hand, no effect on the RFC phenotype is observed if trytophan is injected into *lys rc* larvae. (GRELL, unpublished.)

As stated by JONES and LEWIS (1957) RFC flies homozygous for vermilion do not have pigment in fat cells. However, *v*; *lys rc* animals do have pigmented fat cells (as well as brown eye pigment) if they have been fed kynurenine as larvae (LINDA SMITH RILES, personal communication; GRELL, unpublished). Therefore, kynurenine is a precursor of the fat cell pigment. The enhancement of RFC by starvation may be related to the formation of kynurenine (v^+ substance) when animals with certain alleles of vermilion are starved (BEADLE, TATUM and CLANCY 1938). It is reasonable that nonvermilion animals form more kynurenine when they are starved and therefore more precursor is available for fat cell pigment.

The interaction between the two loci *lys* and *rc* is expected if one considers that each mutation produces a tendency for a RFC phenotype. In single mutants

the tendency must be reinforced by certain environmental conditions, in order to be detectable, but the double mutant expresses the red cells phenotype under all conditions of nutrition and temperature that were tested.

SUMMARY

1. The red fat cells (RFC) phenotype of *Drosophila melanogaster* was found to be a digenic trait. The two recessive mutations are called lysine (*lys*) and red cells (*rc*) and are located at 22.85 and 27.25, respectively, on the second chromosome. These mutations may be separated, maintained in separate stocks, and recombined. When they are recombined, the red cells phenotype is produced again as it was in the original stock.

2. The mutation *lys*, with or without *rc*, causes the accumulation of the amino acid lysine in larvae, pupae and adults. The biochemical basis for the action of *lys* is postulated to be a disturbance in the degradation of lysine. Data to support this hypothesis were obtained from the injection of C^{14}-labeled lysine into *lys* and wild-type flies.

3. Environmental factors alter the RFC phenotype. If the flies develop from starved larvae, *lys* (without *rc*) causes red fat cells. The allele rc^2 likewise produces pigmented cells if the flies were cultured at low temperature or starved.

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ON THE RESISTIBILITY OF DROSOPHILA TO SODIUM CHLORIDE I. STRAIN DIFFERENCE AND HERITABILITY IN D. MELANOGASTER

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IT has been suggested that larvae of Drosophila generally do not require an addition of sodium chloride to the culture media (LOEB 1915; RUBINSTEIN, BURLAKOWA and LWOWA 1935, 1936; KALMUS 1943; KING 1953). Of course this does not mean that they are able to grow in the absence of the sodium salts in the media, because sodium salts are always present in yeasts used for the diet or are included as impurities even in synthetic media. It is generally believed on the other hand that the presence of sodium chloride in excess in the diet exerts an inhibitory effect upon the larval growth and causes eventual death. Recently, IMAIZUMI (unpublished) found that a strain of *D. melanogaster* long reared in our laboratory is highly resistant to the presence of sodium chloride in the culture medium. The author has performed a series of experiments on the resistibility and the requirement of Drosophila for sodium chloride from the genetical and physiological standpoint. The present paper deals with the results of the experiments, in which the strain differences of *D. melanogaster* in the resistibility and the heritability of this character are examined.

MATERIALS AND METHODS

Four strains of brown (*bw*) used were *bw*(Kyoto), *bw*(Pasadena), bw^D and bw^{2b}; of these *bw*(Kyoto) has been reared continuously in our laboratory for many years, and the others were obtained in September 1958 through the courtesy of the California Institute of Technology. Besides the *bw* strains, five wild stocks of Oregon and Canton, namely Ore-RS, Ore-RS(G.23), Ore-R, Ore-R (G.266) and Canton-S were used. Eight mutant strains, sepia (*se*), clot (*cl*), white (*w*), Henna-recessive (Hn^{rd}), vermilion (*v*), *bar-3*(Ives), vestigial (*vg*), and dumpy (*dp*) were also tested. Of these strains, isogenic lines Ore-RS(G.23) and Ore-R(G.266) have been inbred in our laboratory by brother-sister pair matings for 23 and 266 generations respectively before being used. The other strains have been maintained by massmating for many years.

Stocks of these strains have been raised continuously at 25±1°C and under a favorable population density in bottles, (content 180 cc) which contained culture medium consisting of agar 2 g, sugar 6 g, koji (*Aspergillus oryzae* on moist rice) 14 g, KH_2PO_4 0.1 g, water 100 ml; several drops of a thick suspension of yeast added before use.

In order to conduct the present experiments, it was necessary to minimize the NaCl content in the medium. So, instead of the regular culture medium, a semi-synthetic medium according to the following formula was used as the standard: Merck's agar powder 2 g; sugar 10 g; Oriental's dry yeast 10 g; tartaric acid 0.4 g; *n*-butyl benzoate 1 ml (10% solution in 70% ethyl alcohol); and deionized water 100 ml. Butyl benzoate was used to inhibit the growth of molds; tartaric acid was added to the agar solution at temperatures below 70°C to avoid hydrolysis of agar; yeast was killed by boiling.

All experiments were performed at 25°C. To test for resistibility and to select resistant individuals, the NaCl medium was prepared by adding NaCl at a concentration of 1.0 M to the standard medium. At concentrations higher than 1.6 M the medium desiccated soon giving unfavorable effects on the life of flies, and at concentrations lower than 0.3 M strain differences in resistibility were not clearly manifested under the conditions of the present experiments.

Experiment 1: Strain differences in resistibility

Emergence rate test: Twelve mutant and five wild-type strains described above were used in this experiment. Eggs from populations of ten pairs of flies 4–7 days old were transferred to the culture medium within three hours after being laid. Groups of 50 eggs each were put on a glass plate, 2.5 cm × 7.5 cm, which had been covered with 6 g of either the NaCl medium or the standard medium. The plate was introduced into a glass tube 12 cm in length and 3 cm in diameter. A piece of moist filter paper was provided in each tube to give suitable moisture, and the tube was closed with cotton and kept at 25±1°C. Under the experimental conditions described above, the number of emerging adults was counted.

The results are listed in Table 1, in which resistibility is indicated as the proportion of emergence rate in the NaCl medium tests to that in the standard medium tests. As illustrated in the table, the resistibility to NaCl varies in different strains and there seems to exist no correlation between the resistibility and the phenotype of the strain. Although both strains of *bw*(Kyoto) and *bw* (Pasadena) carry the identical *bw* gene, there was a considerable difference between the two strains in resistibility. Similarly, differences in resistibility were found between *bw*(Kyoto) and bw^D and bw^{2b}. Furthermore, the resistibility varied among the five wild-type strains. It is remarkable that Ore-R(G.266) and Ore-RS(G.23) differ from the original strains, Ore-R and Ore-RS, in their resistibilities. Of the 17 strains tested, resistibility was very low in 16 strains: less than 0.1 in 11 strains, and between 0.2 and 0.3 in five strains. The *bw*(Kyoto) strain exhibited a striking contrast to the other strains showing a high resistibility, in which about one half of the eggs grew to imagoes in the NaCl medium, and the resistibility is found to be approximately 0.7.

Emergence rates of *bw*(Kyoto), *se* and Ore-RS on the culture media containing various concentrations of NaCl were examined. As shown in Figure 1, the maximum rate for *bw*(Kyoto) occurred at 0.5 M NaCl, while in Ore-RS and *se* the rate decreased as the concentration of NaCl increased. On the standard medium,

TABLE 1

Emergence rates, resistibilities and sex ratios in strains of D. melanogaster *in the presence of NaCl in the culture medium at a concentration of 1.0 M*

Strain	Date of test	No. of eggs tested		Emergence rate (percent)		Resistibility	Sex ratio	
		In standard medium	In NaCl medium	In standard medium	In NaCl medium		In standard medium	In NaCl medium
bw(Kyoto)	Nov., 1957–Dec., 1958	1000	1000	71.6±4.58	49.2±5.28	0.69±0.039	95.1	78.9
bw(Pasadena)	Nov., 1958–Jan., 1959	500	500	88.6±1.40	27.6±4.00	0.31±0.050	86.1	46.8
Ore-R	Dec., 1958–Oct., 1959	500	500	89.8±0.02	24.4±0.40	0.27±0.007	104.1	90.6
Ore-RS(G.23)	June, 1959	750	750	88.1±5.81	20.3±0.10	0.23±0.015	92.7	76.7
bw^D	May, 1959–Oct., 1959	500	500	90.0±2.80	18.6±1.40	0.21±0.010	90.7	75.5
se	June, 1957–Mar., 1958	750	750	80.3±2.41	14.4±0.86	0.18±0.010	98.0	92.9
Ore-R(G.266)	Dec., 1958	800	800	74.1±2.65	7.1±0.16	0.10±0.007	103.8	128.0
Canton-S	Dec., 1957–Nov., 1959	750	750	89.0±0.60	7.0±1.40	0.08±0.016	96.9	133.3
w	Nov., 1957–Jan., 1960	800	800	86.0±4.57	5.3±0.50	0.06±0.015	90.8	81.8
dp	Dec., 1959–Mar., 1960	750	750	89.4±3.65	4.3±0.26	0.05±0.008	94.0	68.4
v	May, 1959–Jan., 1960	1000	1000	91.8±3.44	4.0±2.04	0.04±0.014	95.2	57.9
bw^{sb}	Nov., 1958–Dec., 1959	750	750	89.9±4.43	3.9±1.36	0.04±0.012	84.2	70.6
bar-3	Dec., 1959–Mar., 1960	750	750	90.6±1.40	3.3±1.85	0.04±0.007	95.9	56.3
Ore-RS	June, 1957–Dec., 1958	750	750	80.7±4.46	3.1±1.70	0.04±0.012	86.2	228.6
cl	Nov., 1957–May, 1958	750	750	75.1±1.61	2.5±1.05	0.03±0.009	90.2	35.7
vg	Dec., 1959–June, 1960	750	750	88.6±2.05	1.1±0.73	0.01±0.007	90.5	60.0
Hn^{rs}	Oct., 1957–May, 1958	750	750	90.9±2.17	0.8±0.61	0.01±0.006	79.5	100.0

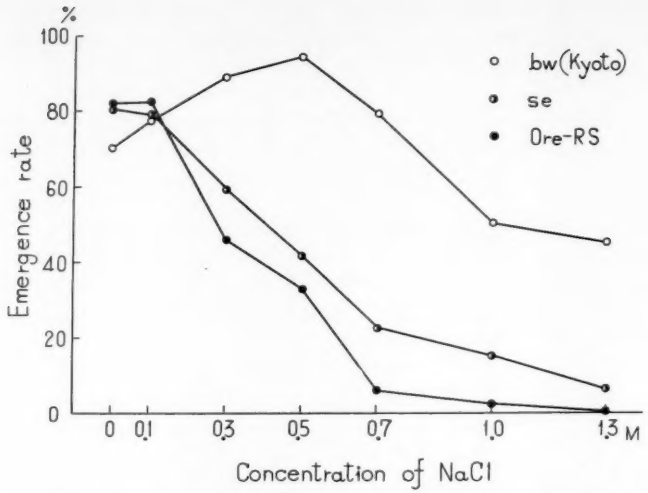

FIGURE 1.—Graph showing emergence rates in three strains of *D. melanogaster* in the culture media containing various concentrations of NaCl.

Ore-RS and *se* strains, both being strongly susceptible to NaCl, showed the highest emergence rate, of about 80 percent. This rate was higher than that of the *bw* (Kyoto) strain by ten percent. At 0.5 M of NaCl, however, the rate dropped to 40 percent in the Ore-RS and *se* strains respectively, while the rate of the *bw* (Kyoto) strain rose to about 95 percent. At 1.3 M, no emerged adult was found in Ore-RS and few in *se*, but the rate of the *bw*(Kyoto) strain remained at one half of the maximum.

The sex ratio of emerged adults seems to be influenced by addition of NaCl to the medium, at least at a concentration of 1.0 M (Table 1). Of the 17 strains tested, 14 strains showed a considerable decrease in number of emerged males as compared with females. On the other hand the ratio of the male exceeded that of the female in the remaining four strains. In the latter strains, however, emerged adults were so few in number as to make the ratios unreliable. This problem will be considered in a separate communication.

The time required for the development is considerably prolonged in the NaCl medium. Under normal conditions at 25°C, the time lapse from hatching to emergence is about 190 hours as reported by BODENSTEIN (1950). With 1.0 M NaCl, it takes at least 220 hours to reach the adult stage. In some cases it took 293 hours, i.e. one and a half times as long as the time in the control.

On a culture medium containing a high concentration of $NaBO_2$ or NaF, emerging adults often show such phenocopies as eyeless, rough and antennaless, or they produce melanotic tumors (SANG and MCDONALD 1954). In the present experiment using NaCl such abnormalities were not found. Adults that emerge on NaCl

medium are somewhat small as compared with flies grown on standard medium, but they are fertile and able to produce progenies quite normal in every respect.

Detailed observations on the effect of the NaCl medium to the developmental processes will be described in a subsequent paper.

Adult mortality test: Two mutant strains *bw*(Koyto), *se* and a wild-type strain Ore-RS were used in this experiment. Flies to be tested were restricted to those of 5–7 days old. About 50 flies, males and females at a ratio of nearly 1:1, were introduced into a milk bottle containing 50 cc of the 1.0 M NaCl medium. The numbers of flies which had died were counted at intervals of 12 hours.

The results obtained were analyzed according to BLISS's method (BLISS 1934, 1937) which had been designed for judging the effect of insecticides. Probit values were obtained from the proportion of dead individuals at the end of each feeding period against the initial number of flies and were plotted in graphic form. Based on this graph, the time at 50 percent mortality (LT-50) and the standard deviation (S) were calculated.

As illustrated in Figure 2, these probit values fall nearly on a straight line. On

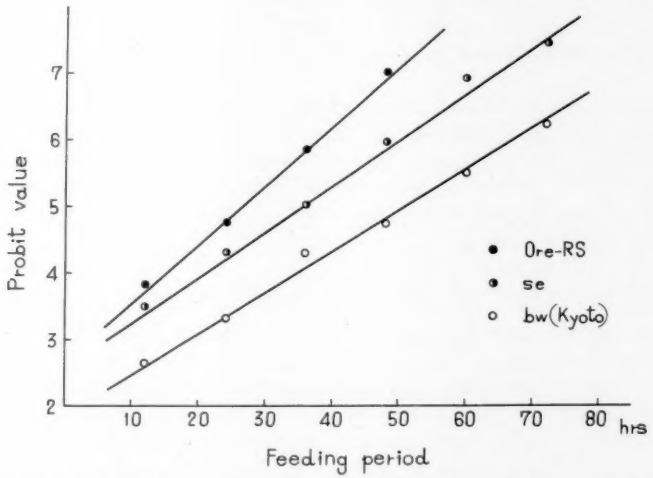

FIGURE 2.—Resistibilities in three strains of *D. melanogaster* to NaCl. Graph showing the probit values of mortalities of adults at each feeding period in the culture medium containing 1.0 M NaCl.

the NaCl medium, about one half of the flies in the Ore-RS strain died within 24 hours from initiation of the experiment, while in the *se* and *bw*(Kyoto) strains the mortalities within 24 hours were less than 25 percent and five percent respectively. Striking contrasts are afforded at the end of the 60th hour in that there were observed no survivors in Ore-RS and only four percent in *se* while 30 percent of the *bw*(Kyoto) tested were still alive. Even in the resistant *bw*(Kyoto)

strain, however, no survivor was found on the NaCl medium after 96 hours. In contrast, there hardly appears in any strain any fly mortally weakened during the course of this experiment in the standard medium. As presented in Table 2,

TABLE 2

Resistibilities and sex ratios of adults in three strains of D. melanogaster *to NaCl. In the standard medium hardly any fly in any one of the strains died within 96 hours from initiation of the experiment*

Strain	No. of flies tested ♂	♀	LT-50 (hours)	S	Sex ratio of survivors at LT-50 ♂ : ♀
bw(Kyoto)	1374	1299	51.5	16.95	77.6:100
se	1440	1455	36.0	14.08	84.8:100
Ore-RS	1393	1317	26.5	10.75	61.8:100

LT-50 in the NaCl medium was 26.5 hours for Ore-RS, 36.0 hours for *se*, and 51.5 hours for *bw*(Kyoto). From these results, it is clear that flies of the *bw* (Kyoto) strain are most resistant to NaCl among the three strains, and that the *se* strain is more resistant than Ore-RS. These facts are well in accord with the resistibilities of these strains as manifested in the emergence rate test mentioned above.

The sex ratio of *D. melanogaster* is generally believed to be 90–95 males against 100 females in normal cultures. As far as these strains are concerned, the sex ratio of survivors at LT-50 is shifted considerably from that of the control by an addition of 1.0 M NaCl to the medium. The ratio of female to male is increased (Table 2). These results would suggest that in the adult stage the female is more resistant to NaCl than the male.

Experiment 2: Increase of resistibility to NaCl by selection

In order to determine whether the resistibility to NaCl is a hereditary character, the *bw*(Kyoto) strain which revealed the highest resistibility (0.69) in the preceding experiment, was reared through successive generations on 1.0 M NaCl medium. At the initiation of the test, 500 eggs laid by populations of ten pairs of flies randomly chosen were put on the 1.0 M NaCl medium and left to grow to imagoes under the same culture conditions as used in experiment 1. The emerged adults were counted and the rate calculated. Under these conditions much fewer eggs were laid than under normal culture conditions. To obtain a sufficient number of eggs for continuation of the selection experiment, the survivors were transferred to the standard medium at the end of the 24th hour after emergence. In successive generations, eggs laid on this standard medium were transferred to 1.0 M NaCl medium and reared as in the first generation. Thus resistant individuals were selected from generation to generation using the NaCl medium. The emergence rate on the standard medium was tested in some generations to permit comparisons with that on the NaCl medium. While continuing the selection along the line of generations with the NaCl medium (line A in Figure 3), two lines

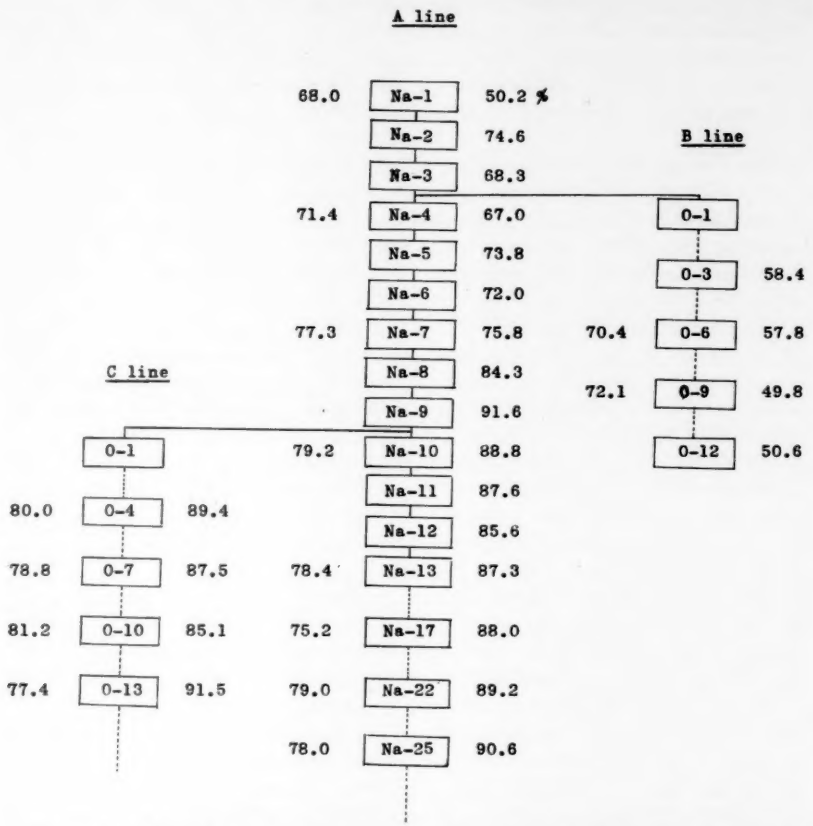

FIGURE 3.—Increase of the resistibility to NaCl by selection in *bw*(Kyoto) strain of *D. melanogaster*. Figures in the box of A line are the generation number of the flies reared in the culture medium containing 1.0 M NaCl. Those of B and C lines are the generation number of the flies in the standard medium without addition of NaCl. A value on the right of each box shows emergence rate in the NaCl medium, and a value on the left shows the rate in the standard medium. All values are based on 500 eggs.

were separated from the original line; one was started at the third generation (line B), and the other at the ninth generation (line C). The members of these latter lines were reared on the standard medium from generation to generation. In each generation the emergence rate in the NaCl medium was tested in order to determine whether the resistibility raised by selection in the original line could be maintained in the absence of selection pressure.

The emergence rates in the three lines are illustrated in Figure 3. In the initial generation, the emergence rate was 50.2 percent in the NaCl medium and 68.0

percent in the standard medium. In the generations of line A, the rate in NaCl medium rose gradually to 91.6 percent in the ninth generation, i.e. higher than in the standard medium. It remained almost unchanged in subsequent generations, at least up to the 25th generation. These facts would indicate that selection pressure is very effective in raising the resistibility to the maximum attained after nine generations. The rate in the standard medium also increased but more slowly through the generations in this line. In the 25th generation the rate was 78.0 percent, being higher than that in the initial generation by ten percent. The emergence rates both in the NaCl and in the standard medium ascended with the succession of generations. The experiment is being continued in order to examine whether the resistibility is remaining at its high level in subsequent generations.

Line B, started from the eggs produced by flies of the third generation in line A, was raised on the standard medium. In line A the emergence rate in the NaCl medium had at that generation been raised to 68.3 percent, while no remarkable change had occurred yet with regard to the rate in the standard medium. The resistibility to NaCl in line B decreased from generation to generation and returned to the level of the initial strain within eight generations. Then it remained unchanged through subsequent generations at least up to the 12th generation. The emergence rate in the standard medium, on the other hand, was maintained practically at the initial level through these generations. The experiment using line B was discontinued at the 12th generation.

Line C was derived from the ninth generation of line A. It exhibited a striking contrast to line B with regard to the stability of the emergence rates in NaCl and in standard medium. In the ninth generation of line A, the emergence rates in the NaCl medium and in the standard medium were as high as the maximum rates of the line. Through 13 successive generations of line C, these rates persisted with only slight fluctuations. It should be noted that the high rate was kept nearly unchanged at least through 13 generations, notwithstanding the fact that flies in this line had not been exposed to the NaCl medium but had been reared exclusively on standard medium. From the results of the selection experiment, it is concluded that lines C and A were stabilized with regard to the resistibility to NaCl. Another noteworthy fact is that the emergence rate in the standard medium remained always lower than that in the NaCl medium by about ten percent.

DISCUSSION

The role of NaCl in the diet of Drosophila is not well understood. Loeb (1908) was able to raise larvae of *D. melanogaster* in the absence of NaCl except as impurities in the chemicals used. This experiment of Loeb was confirmed by Rubinstein, Lwowa and Burlakowa (1935, 1936). Kalmus (1943) also showed that *D. melanogaster* could be reared without addition of NaCl to the diet. Sang's synthetic culture medium (1956) contained only a trace of NaCl. These data would suggest that *D. melanogaster* hardly require, if at all, the presence of NaCl in the diet. A large amount of NaCl is definitely deleterious.

Thus, in the recent work by KING (1953), who studied the effects of alkali metal ions on the development of *D. melanogaster*, a highly inbred Canton-S strain did not grow in a culture medium containing 0.5 M NaCl. This result seems to be in accord with the general belief that an excess of sodium chloride in the diet causes inhibition of growth and eventual death. An exception to this is the recent result of WADDINGTON (1959), in which Ore-K showed emergence rate of about 50 percent in a culture medium containing six percent NaCl (about 1 M), the rate being as high as the level of *bw*(Kyoto) strain tested by the writer.

Sodium salts are generally known to affect osmotic regulation of living matters. Data given by WIGGLESWORTH (1938) show the average osmotic pressure of insect haemolymph to be approximately equivalent to 0.18 M NaCl. In larvae of Diptera, it is equivalent to 0.23 M NaCl at a maximum and 0.10 M at a minimum. Taking these dates into account, the concentration of NaCl in the culture medium of the present work would be several times as high as that isosmotic with Drosophila haemolymph. The NaCl medium may exert some hyperosmotic effects upon the internal organs when taken into the digestive tract, and afford a desiccating environment to Drosophila through its life span. Recently, CROGHAN and LOCKWOOD (1960) reported that the haemolymph of larvae of Ore-K developing in a medium containing seven percent NaCl was markedly hypotonic to the medium and that there was only a small rise of the NaCl concentration in the haemolymph. WADDINGTON (1959) reported that the anal papilla of the fly resistant to such high concentration of NaCl is larger in size than that of the susceptible one. The function of this organ is not known exactly but is supposed to be concerned with osmotic regulation. In the present material, however, no morphological changes were noticed.

In the present work the resistibility to NaCl attained by the strain selected through continual application of the NaCl medium is higher than among any other known strains. The selection experiment proves the character to be hereditary. The attainment of this resistibility would be deduced to have been the consequence of selection pressure exerted through successive generations in the presence of NaCl at a high concentration in the culture medium rather than due to the result of a built-up tolerance in individuals following successive NaCl intake. Therefore, the name srb (sodium chloride resistant brown) is given to the selected strain which is stable with regard to the high resistibility to NaCl. The raised resistibility of the strain is maintained at nearly constant level through many subsequent generations, reared on normal culture medium which does not contain a detectable amount of NaCl. In addition to the increase in resistibility to NaCl, emergence rate in the standard medium also rises slowly but steadily in line A. One may assume that some selection pressure toward higher emergence rate is active in the standard medium.

It is very interesting that the presence of high concentrations of NaCl in the medium is found to furnish a more favorable environment to both the *bw*(Kyoto) and srb strains than does the medium without NaCl. From this fact alone, however, it cannot be concluded that these strains have a requirement for NaCl. To

solve the problem whether the high resistibility to NaCl in these strains is related to such a requirement further work is necessary.

To elucidate the nature of hereditary resistibility of Drosophila to NaCl additional experiments are under way.

SUMMARY

(1) The present paper deals with various phenomena revealed by many strains of *Drosophila melanogaster* under the presence of NaCl added to the culture medium.

(2) The resistibility to NaCl varies in different strains, and there is no correlation between resistance and the mutant markers or lack of markers (Visibles) in the various strains.

(3) Only about one to 28 percent of the eggs from most strains grew to imagoes in a medium containing 1.0 M NaCl. The *bw*(Kyoto) strain exhibited a striking contrast to other strains by its high resistibility, in which about one half of the eggs grew to imagoes in the medium containing 1.0 M NaCl. The most remarkable difference is seen in the adult mortality test: within 60 hours on the medium, all adults of the *se* and Ore-RS strains had died, while 30 percent of *bw*(Kyoto) survived.

(4) The high resistibility is not a general character of different *bw* strains.

(5) The sex ratio of emerged adults in both resistant and susceptible strains seems to be influenced, at least at a concentration of 1.0 M NaCl.

(6) The resistibility to NaCl is a hereditary character. By raising the *bw* (Kyoto) strain through successive generations on 1.0 M NaCl medium, it was possible to raise the emergence rate gradually to 91.6 percent at the ninth generation after which it remained almost unchanged, at least up to the 25th generation. The increased resistibility of the strain was maintained through many subsequent generations at nearly the same level even after culture on normal NaCl "free" medium.

Throughout the text "emergence rate" does not mean number emerging per unit time but a simple percent emergence.

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SOME GENERAL FORMULATIONS OF LINKAGE EFFECTS IN INBREEDING

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THE effect of a given system of consanguineous mating on the degree of homozygosity of resulting individuals is conveniently described by means of the coefficient of inbreeding (WRIGHT 1921). The coefficient of inbreeding, F, of an individual may be defined to be the probability that the two genes possessed by that individual at a locus are identical by descent (MALÉCOT 1948; KEMPTHORNE 1957). The probability that with respect to a given set of n loci the two gametes of an individual are occupied throughout by pairs of genes identical by descent is equal to F^n provided that the loci in question are not linked. A more general formulation of this probability, allowing for arbitrary linkages, is needed in certain problems of population genetics. Such problems include the interpretation of covariances between relatives (COCKERHAM 1956). The present article gives several relations which may be useful for formulating and applying the required generalization of the coefficient of inbreeding.

Gametic frequencies and recombination values: We shall have to employ recombination values as well as frequencies of modes of gamete formation. Therefore any general connection between those two categories of probabilities would be helpful. It has been stated by several writers (see for example GEIRINGER 1944, OWEN 1950) that the gametic frequencies cannot be expressed entirely as linear functions of recombination values, if the number of loci involved is greater than three. This obstacle, however, can be surmounted by introducing new classes of recombination values.

Consider four linked loci, i, j, k, l, arranged in this order on the chromosome. A diploid individual produces $2^4 = 16$ different types of gametes with respect to those loci. The probability that the individual transmits to its progeny a certain gamete will be denoted by γ followed by a four-digit subscript, where the digits refer to the corresponding loci in the sequence given above. The figures 0 and 1 will be used to distinguish the two cases that a locus carries the maternal or the paternal gene, respectively. Thus γ_{0100} is the probability that a transmitted gamete contains the maternal genes at loci i, k, l, and the paternal gene at locus j. Recombination values will be denoted by ρ with subscripts specifying the loci involved, i.e. ρ_{ij}, etc.

Since the gametic frequencies must add to unity, and owing to obvious sym-

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metry relations such as $\gamma_{0000} = \gamma_{1111}$, $\gamma_{1000} = \gamma_{0111}$, etc., the 16 frequencies of types of gametes can be specified by means of seven parameters. If the gametic frequencies are known, each recombination value can be found. For instance,

$$\rho_{ij} = \gamma_{1000} + \gamma_{0100} + \gamma_{1010} + \gamma_{1001} + \gamma_{0110} + \gamma_{0101} + \gamma_{1011} + \gamma_{0111}.$$

As we have six recombination values, there are six equations of the form exemplified above, which are not solvable for the gametic frequencies. In order to reveal the one additional parameter needed we should think of segments rather than of loci. Let u, v, w be the chromosomal segments between loci i and j, j and k, k and l, respectively. Then the six recombination values available are as follows:

ρ_{ij} referring to the segment u,
ρ_{jk} referring to the segment v,
ρ_{kl} referring to the segment w,
ρ_{ik} referring to the compound segment $(u + v)$,
ρ_{jl} referring to the compound segment $(v + w)$,
ρ_{il} referring to the compound segment $(u + v + w)$.

It is easily seen that a recombination value referring to the compound segment $(u + w)$ is wanted in order to balance the system. Let us denote that quantity by ρ_{ijkl} and define it in analogy to other recombination values which refer to the compound of two segments. Thus, ρ_{ijkl} is defined to be the sum of the probabilities of two mutually exclusive events, *viz.* (a) an odd number of crossovers in u, conjunctive with an even number in w, and (b) an even number in u, conjunctive with an odd number in w. In terms of gametic frequencies, we may write,

$$\rho_{ijkl} = \gamma_{1000} + \gamma_{0100} + \gamma_{0010} + \gamma_{0001} + \gamma_{1110} + \gamma_{1101} + \gamma_{1011} + \gamma_{0111}. \qquad (1)$$

Using the quantity so defined we can express each gametic frequency as a linear function of the seven recombination values now available. Pairs of gametic frequencies which are equal owing to symmetry relations may be summed to obtain the eight frequencies of modes of gamete formation listed in Table 1. The

TABLE 1

Frequencies of modes of gamete formation expressed as linear functions of recombination values, for four loci involved

$(\gamma_{0000} + \gamma_{1111}) =$	$1 - \frac{1}{4}\rho_{ij} - \frac{1}{4}\rho_{ik} - \frac{1}{4}\rho_{il} - \frac{1}{4}\rho_{jk} - \frac{1}{4}\rho_{jl} - \frac{1}{4}\rho_{kl} - \frac{1}{4}\rho_{ijkl}$
$(\gamma_{1000} + \gamma_{0111}) =$	$\frac{1}{4}\rho_{ij} + \frac{1}{4}\rho_{ik} + \frac{1}{4}\rho_{il} - \frac{1}{4}\rho_{jk} - \frac{1}{4}\rho_{jl} - \frac{1}{4}\rho_{kl} + \frac{1}{4}\rho_{ijkl}$
$(\gamma_{0100} + \gamma_{1011}) =$	$\frac{1}{4}\rho_{ij} - \frac{1}{4}\rho_{ik} - \frac{1}{4}\rho_{il} + \frac{1}{4}\rho_{jk} + \frac{1}{4}\rho_{jl} - \frac{1}{4}\rho_{kl} + \frac{1}{4}\rho_{ijkl}$
$(\gamma_{0010} + \gamma_{1101}) =$	$-\frac{1}{4}\rho_{ij} + \frac{1}{4}\rho_{ik} - \frac{1}{4}\rho_{il} + \frac{1}{4}\rho_{jk} - \frac{1}{4}\rho_{jl} + \frac{1}{4}\rho_{kl} + \frac{1}{4}\rho_{ijkl}$
$(\gamma_{0001} + \gamma_{1110}) =$	$-\frac{1}{4}\rho_{ij} - \frac{1}{4}\rho_{ik} + \frac{1}{4}\rho_{il} - \frac{1}{4}\rho_{jk} + \frac{1}{4}\rho_{jl} + \frac{1}{4}\rho_{kl} + \frac{1}{4}\rho_{ijkl}$
$(\gamma_{1100} + \gamma_{0011}) =$	$-\frac{1}{4}\rho_{ij} + \frac{1}{4}\rho_{ik} + \frac{1}{4}\rho_{il} + \frac{1}{4}\rho_{jk} + \frac{1}{4}\rho_{jl} - \frac{1}{4}\rho_{kl} - \frac{1}{4}\rho_{ijkl}$
$(\gamma_{1010} + \gamma_{0101}) =$	$\frac{1}{4}\rho_{ij} - \frac{1}{4}\rho_{ik} + \frac{1}{4}\rho_{il} + \frac{1}{4}\rho_{jk} - \frac{1}{4}\rho_{jl} + \frac{1}{4}\rho_{kl} - \frac{1}{4}\rho_{ijkl}$
$(\gamma_{1001} + \gamma_{0110}) =$	$\frac{1}{4}\rho_{ij} + \frac{1}{4}\rho_{ik} - \frac{1}{4}\rho_{il} - \frac{1}{4}\rho_{jk} + \frac{1}{4}\rho_{jl} + \frac{1}{4}\rho_{kl} - \frac{1}{4}\rho_{ijkl}$

expressions can be seen to be equivalent to those given by OWEN (1950) for the four-loci case, if his function "w" is specified by

$$\text{"}w\text{"} = \tfrac{1}{4}(\rho_{ij} - \rho_{ik} + \rho_{il} + \rho_{jk} - \rho_{jl} + \rho_{kl} - \rho_{ijkl}).$$

The quantity ρ_{ijkl} should be recognized to be a true recombination value, differing from the customary ones mainly in that it refers to a compound of non-

contiguous intervals. Since it contains information not furnished by the other recombination values, it may well prove to be useful also in such problems as chromosome mapping and deriving laws of chiasma formation. In any case, a complete description of four-point linkage data should include an estimate of ρ_{ijkl}. We note that ρ_{ijkl} is directly observable just as ordinary recombination values, without any knowledge of order of loci. In fact, whichever order of loci might erroneously be assumed in identifying the chromosomal segments, defining ρ_{ijkl} as above with respect to the presumed first and third segment leads always to the same quantity specified in (1). It is this property of ρ_{ijkl} which makes the relations given in Table 1 universally applicable also to those cases when not all the loci involved are situated on the same chromosome. It may be mentioned that ρ_{ijkl} is equal to ½ if at least one of the two pairs of loci, *i–j* and *k–l*, is not linked. But if the loci *j–k* are not linked, ρ_{ijkl} has the value $(\rho_{ij} + \rho_{kl} - 2\rho_{ij}\rho_{kl})$, as it should have with zero interference.

The generalization to n loci is straightforward. There are $\frac{1}{2}n(n-1)$ customary recombination values available, while $(2^{n-1} - 1)$ parameters are necessary for specifying 2^n gametic frequencies. Hence the number of additional recombination values is $\frac{1}{2}(2^n - 2 - n^2 + n)$. With five loci, *i*, *j*, *k*, *l*, *m*, the ten customary values, $\rho_{ij}, \rho_{ik}, \ldots, \rho_{lm}$, can be completed by five values of the sort introduced above, *viz.* $\rho_{ijkl}, \rho_{ijkm}, \rho_{ijlm}, \rho_{iklm}$, and ρ_{jklm}. With six or more loci we meet new classes of recombination values, which refer to the compounds of three or more noncontiguous intervals, showing in all other respects complete analogy to the classes of recombination values already known. As the order of loci is irrelevant, any recombination value referring to s noncontiguous segments can univocally be denoted by specifying the set of $2s$ loci involved in confining those segments. Then with n loci we shall have,

- $\binom{n}{2}$ two-loci recombination values like ρ_{ij},
- $\binom{n}{4}$ four-loci recombination values like ρ_{ijkl},
- $\binom{n}{6}$ six-loci recombination values like ρ_{ijklmp}, etc.,

or generally, $\binom{n}{2s}$ $2s$-loci recombination values, where s runs over all integers from 1 to z, with $z = \frac{1}{4}[2n - 1 + (-1)^n]$. Since

$$\sum_{s=1}^{z}\binom{n}{2s} = 2^{n-1} - 1,$$

it holds true that for any number of loci, the number of recombination values, summed over all classes, is equal to the number of parameters needed to specify the appropriate frequencies of types of gametes.

Gametic frequencies and linkage values: In applying the relations indicated above it will often pay to replace recombination values by a complementary system of parameters, which may be called *linkage values*. For each recombination value, ρ, a corresponding linkage value, λ, can be defined by means of the general relation,

$$\lambda = 1 - 2\rho. \tag{2}$$

Note that the quantity λ is unity with complete linkage, and zero with linkage being absent. A linkage value may only be negative if the corresponding recombination fraction exceeds 50 percent.

Expressions of the gametic frequencies in terms of appropriate linkage values form completely orthogonal systems for any number of loci involved. For example, we have with respect to two loci,

$$\gamma_{00} = \gamma_{11} = \tfrac{1}{4}(1 + \lambda_{ij})$$
$$\gamma_{10} = \gamma_{01} = \tfrac{1}{4}(1 - \lambda_{ij})$$

and, referring to three loci,

$$\gamma_{000} = \gamma_{111} = \tfrac{1}{8}(1 + \lambda_{ij} + \lambda_{ik} + \lambda_{jk})$$
$$\gamma_{100} = \gamma_{011} = \tfrac{1}{8}(1 - \lambda_{ij} - \lambda_{ik} + \lambda_{jk})$$
$$\gamma_{010} = \gamma_{101} = \tfrac{1}{8}(1 - \lambda_{ij} + \lambda_{ik} - \lambda_{jk})$$
$$\gamma_{001} = \gamma_{110} = \tfrac{1}{8}(1 + \lambda_{ij} - \lambda_{ik} - \lambda_{jk}).$$

In the four-loci case the frequencies are:

$$\gamma_{0000} = \gamma_{1111} = \tfrac{1}{16}(1 + \lambda_{ij} + \lambda_{ik} + \lambda_{il} + \lambda_{jk} + \lambda_{jl} + \lambda_{kl} + \lambda_{ijkl})$$
$$\gamma_{1000} = \gamma_{0111} = \tfrac{1}{16}(1 - \lambda_{ij} - \lambda_{ik} - \lambda_{il} + \lambda_{jk} + \lambda_{jl} + \lambda_{kl} - \lambda_{ijkl})$$
$$\gamma_{0100} = \gamma_{1011} = \tfrac{1}{16}(1 - \lambda_{ij} + \lambda_{ik} + \lambda_{il} - \lambda_{jk} - \lambda_{jl} + \lambda_{kl} - \lambda_{ijkl})$$

and so on, the residual equations being easily written down from Table 1. The term "unity", which stands after the first bracket in each of the foregoing expressions, may be interpreted as being an additional linkage value referring to zero loci. Such a linkage value, which in case of necessity may be symbolized by $\lambda_{()}$, must always be equal to unity since recombination is impossible with zero loci. Among other things, the proposed interpretation has the advantage of shortening general formulations.

In order to facilitate generalization we shall also employ the following notation: Variable sets of loci are symbolized by capital letters, while the number of loci included in such a set is denoted by the corresponding lower case letter. The sign $\subset$ means "contained in". For instance, the condition $Q \subset R$ states that set Q does not involve any locus which is not comprised within set R, or in other words, that Q is a subset of set R. The remaining subset, $(R - Q)$, is referred to as $\overline{Q}$. The sign $\sum_{Q \subset R}$ indicates summation over all possible subsets Q within a particular set R, i.e. over 2^r items, as there are 2^r different ways of dividing set R into two subsets, Q and $\overline{Q}$. Furthermore, we denote by MQ that subset of loci which is common to two particular sets, M and Q. The number of loci involved in subset MQ is symbolized by $c(MQ)$. Finally, a capital followed by an asterisk, such as M^*, refers only to those subsets in which the number of loci is either even or zero.

Making use of the above notation, we denote by $\gamma_{R(Q)}$ the frequency of that type of gamete which carries maternal genes at the loci of subset Q, and paternal genes at the loci of subset $\overline{Q}$, so that $(Q + \overline{Q}) = R$. Similarly, the recombination value which can be defined by means of a certain subset of loci, M^*, will be written in the general form ρ_{M^*}, and λ_{M^*} will stand for the corresponding linkage value. Then the general definition of the linkage values in terms of gametic frequencies is given by the equation.

$$\lambda_{M^*} = \sum_{Q \subset R} (-1)^{c(M^*Q)} \gamma_{R(Q)}, \tag{3}$$

where R may be any set of loci chosen in such a way that it comprehends the particular set M^* as a subset, i.e. that $M^* \subset R$. The general definition of the

recombination values is obtainable by combining formulae (2) and (3). Regarding the gametic frequencies, expressions in terms of appropriate linkage values are provided by the general relation,

$$\gamma_{R_{(Q)}} = (\tfrac{1}{2})^r \sum_{M^* \subset R} (-1)^{c(M^*Q)} \lambda_{M^*}. \tag{4}$$

With respect to the special case $r = 0$, formula (4) implies the existence of one gametic frequency which is equal to unity. In the case $r = 1$ we have, of course, $\gamma_0 = \gamma_1 = \tfrac{1}{2}$.

Generalization of the coefficient of inbreeding: We are concerned with the probability that the two gametes possessed by an individual are identical by descent regarding a given set of loci symbolized by R. Let ϕ_R be that probability. Apparently the value of ϕ_R is influenced by three things, *viz.* (a) the system of inbreeding employed in giving rise to the individual in question, (b) the number of loci considered, r, and (c) the specific linkage relations existing between those loci. The information as to the two latter points is contained in full by the set of gametic frequencies which is characteristic of R. Therefore ϕ_R can be given as a function of the gametic frequencies belonging to set R, the type of that function depending on the inbreeding system employed.

For a simple example, let us consider an individual Z which has arisen from selfing a noninbred parent X. With regard to a given set of loci, R, the two gametes of individual Z will be identical by descent if, and only if, both of them have originated from the same type of gamete of individual X. Hence in this case the probability ϕ_R can be found by squaring the gametic frequencies belonging to set R and summing. Writing ϕ with subscripts referring to the loci involved, we have,

$$\begin{aligned}
\phi_i &= \gamma_0^2 + \gamma_1^2 = \tfrac{1}{2}, \\
\phi_{ij} &= \gamma_{00}^2 + \gamma_{10}^2 + \gamma_{01}^2 + \gamma_{11}^2 = \tfrac{1}{4}(1 + \lambda_{ij}^2), \\
\phi_{ijk} &= \tfrac{1}{8}(1 + \lambda_{ij}^2 + \lambda_{ik}^2 + \lambda_{jk}^2), \\
\phi_{ijkl} &= \tfrac{1}{16}(1 + \lambda_{ij}^2 + \lambda_{ik}^2 + \lambda_{il}^2 + \lambda_{jk}^2 + \lambda_{jl}^2 + \lambda_{kl}^2 + \lambda_{ijkl}^2),
\end{aligned} \tag{5}$$

and so on, or generally,

$$\phi_R = \sum_{Q \subset R} \gamma_{R_{(Q)}}^2 = (\tfrac{1}{2})^r \sum_{M^* \subset R} \lambda_{M^*}^2. \tag{6}$$

General expressions of the probability ϕ for more intricate systems of inbreeding are necessarily more difficult, and sometimes even prohibitive because of complexity. Nevertheless, ϕ_R is always a function involving no other unknowns than the set of gametic frequencies belonging to R, and for any given system of inbreeding the type of that function will be the same for all sets R, irrespective of the number of loci involved. In view of this fact we may term the probability ϕ as *function of inbreeding*. Being a generalization of WRIGHT's coefficient of inbreeding, ϕ_R is equal to F for the case $r = 1$. It is only with $r \geqq 2$ that linkage may influence the value of ϕ_R, the effect being nearly always to increase ϕ_R above F^r, which is the value of ϕ_R without linkage. As to the case $r = 0$, it can be seen to be a necessary assumption that $\phi_R = 1$, whether inbreeding of any kind has been employed or not.

Gametic frequencies and linkage values are qualified in different ways for expressing the function of inbreeding. The γ-system of parameters is best suited

for specifying the type of an inbreeding function, but has the disadvantage of giving no indication of its actual value in any given instance. On the other side, the λ-system of parameters seems to be less handy for general formulations, but for sets of two or three loci at least it furnishes rather simple formulae of ϕ_R, separating that component of ϕ_R that is due to linkage. Therewith it becomes possible to compare different inbreeding pedigrees as to their effects on certain values of ϕ_R. For the purpose of illustration we shall consider the three pedigrees shown in Figures 1–3, all leading to the same coefficient of inbreeding, *viz.* $F = 1/4$.

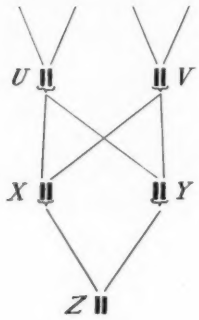

Figure 1

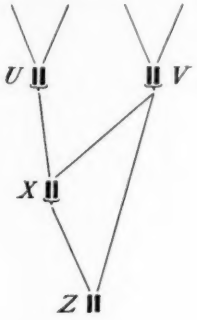

Figure 2

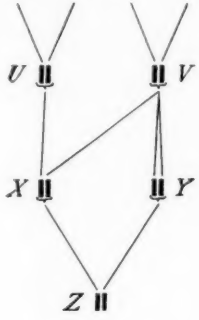

Figure 3

FIGURE 1.—Inbreeding by mating full sibs. FIGURE 2.—Inbreeding by mating parent to offspring. FIGURE 3.—Inbreeding by mating two sibs one of which has arisen from selfing.

In the first example (Figure 1) the inbred individual, Z, results from mating two full sibs, X and Y, which on their turn have individuals U and V as the common female and male parent, respectively. The type of the inbreeding function can be derived from the two-loci case by means of Table 2. The headings

TABLE 2

Auxiliary table for deriving the function of inbreeding for the pedigree shown in Figure 1 (for explanation see text)

X \ Y	γ_{00}	γ_{10}	γ_{01}	γ_{11}
γ_{00}	$(\gamma_{00}^2+\gamma_{10}^2+\gamma_{01}^2+\gamma_{11}^2)\gamma_{..}^2$	.....	.....	.....
γ_{10}	.....	$(\gamma_{.0}^2+\gamma_{1.}^2)(\gamma_{0.}^2+\gamma_{1.}^2)$	.....	.....
γ_{01}	.....	.....	$(\gamma_{0.}^2+\gamma_{1.}^2)(\gamma_{.0}^2+\gamma_{.1}^2)$	.....
γ_{11}	.....	.....	.....	$\gamma_{..}^2(\gamma_{00}^2+\gamma_{10}^2+\gamma_{01}^2+\gamma_{11}^2)$

of rows and columns of the table specify the frequencies of types of gametes formed by individuals X and Y, respectively. Each cell gives the probability that the two gametes referred to in the marginal headings of that cell are identical by descent. Dots replacing digits in the subscript of a gametic frequency indicate summation over the digits 0 and 1 at the respective loci. For instance, $\gamma_{\cdot 0}$ stands for $(\gamma_{00} + \gamma_{10})$. Note that each of the probabilities in the diagonal is the product of two expressions of gametic frequencies which refer to the gametes transmitted by individuals U and V, respectively. The function of inbreeding is found by multiplying the probability of each cell with the product of its two marginal gametic frequencies, and summing. Expressing the result in a form, which is general for r loci, we may write the type of the function of inbreeding as

$$\phi_R = \sum_{Q \subset R} \gamma^2_{R_{(Q)}} \sum_{H \subset Q} \gamma^2_{Q_{(H)}} \sum_{G \subset \overline{Q}} \gamma^2_{\overline{Q}_{(G)}} \tag{7}$$

$$= (\tfrac{1}{2})^{3r} \sum_{Q \subset R} \Big[\sum_{M^\bullet \subset R} (-1)^{c(M^\bullet Q)} \lambda_{M^\bullet} \Big]^2 \sum_{L^\bullet \subset Q} \lambda^2_{L^\bullet} \sum_{K^\bullet \subset \overline{Q}} \lambda^2_{K^\bullet}.$$

From formula (7) the value of ϕ_R can be written down for any given number of loci, if wanted. While the formulae become unwieldy for $r > 2$, we have for $r = 1$,

$$\phi_i = F = (\tfrac{1}{2})^2(\tfrac{1}{2})(1) + (\tfrac{1}{2})^2(1)(\tfrac{1}{2}) = \tfrac{1}{4},$$

and for $r = 2$, (8)

$$\phi_{ij} = (\tfrac{1}{4} + \tfrac{1}{4}\lambda_{ij})^2(\tfrac{1}{4} + \tfrac{1}{4}\lambda^2_{ij})(1) + (\tfrac{1}{4} - \tfrac{1}{4}\lambda_{ij})^2(\tfrac{1}{2})(\tfrac{1}{2}) + (\tfrac{1}{4} - \tfrac{1}{4}\lambda_{ij})^2(\tfrac{1}{2})(\tfrac{1}{2}) + (\tfrac{1}{4} + \tfrac{1}{4}\lambda_{ij})^2(1)(\tfrac{1}{4} + \tfrac{1}{4}\lambda^2_{ij})$$

$$= \tfrac{1}{16}(1 + \tfrac{3}{2}\lambda^2_{ij} + \lambda^3_{ij} + \tfrac{1}{2}\lambda^4_{ij}).$$

The next pedigree, shown in Figure 2, concerns inbreeding by parent-offspring mating. Denoting by $\gamma_{R_{()}}$ the frequency of that type of gamete which transmits paternal genes at all the loci of set R, we may formulate the type of the function of inbreeding as

$$\phi_R = \gamma_{R_{()}} \sum_{Q \subset R} \gamma^2_{R_{(Q)}} = (\tfrac{1}{2})^{2r} \sum_{M^\bullet \subset R} \lambda_{M^\bullet} \sum_{L^\bullet \subset R} \lambda^2_{L^\bullet} \tag{9}$$

As to special cases, we have for $r = 1$,

$$\phi_i = \tfrac{1}{4},$$

and for $r = 2$, (10)

$$\phi_{ij} = \tfrac{1}{16}(1 + \lambda_{ij} + \lambda^2_{ij} + \lambda^3_{ij}).$$

In the last pedigree to be considered here (Figure 3) the inbred individual, Z, results from mating two sibs one of which has arisen from selfing. The type of the appropriate function of inbreeding is expressible as

$$\phi_R = \gamma_{R_{()}} \sum_{Q \subset R} \gamma_{R_{(Q)}} \sum_{M \subset R} \gamma_{R_{(M)}} \gamma_{M_{(MQ)}} \gamma_{\overline{M}_{(\overline{M}Q)}} \tag{11}$$

A corresponding expression in terms of linkage values is too cumbersome for mentioning. For the case $r = 1$ we find again,

$$\phi_i = \tfrac{1}{4},$$

whereas the case $r = 2$ comes out with (12)

$$\phi_{ij} = \tfrac{1}{16}(1 + \lambda_{ij} + \tfrac{1}{2}\lambda^2_{ij} + \lambda^3_{ij} + \tfrac{1}{2}\lambda^4_{ij}).$$

It is of interest to compare the value of ϕ_{ij} given in (12) with the corresponding expressions in (8) and (10) for full-sib mating, and parent-offspring mating,

respectively. Regarding the former case, the value of ϕ_{ij} in (12) surpasses that one reached in (8) by the amount of $\frac{1}{16}(\lambda_{ij} - \lambda_{ij}^2)$. As to the latter case, the value of ϕ_{ij} in (12) is smaller than that found in (10) by the amount of $\frac{3}{32}(\lambda_{ij}^2 - \lambda_{ij}^4)$. Both quantities will be either zero or positive (except λ_{ij} is negative, which is not likely to be of frequent occurrence). Thus, inbreeding by mating two sibs one of which has arisen from selfing appears to be intermediate between full-sib inbreeding and parent-offspring inbreeding with regard to the effect of linkage on the value of ϕ_{ij}. This result will be extended in the last section.

Generalization of the panmictic index: Inbreeding theory sometimes makes use of WRIGHT's panmictic index, P, which is the complement of the coefficient of inbreeding, i.e. $P = 1 - F$. We may generalize the panmictic index in the same way as it has been done for the coefficient of inbreeding in the preceding section. Let π_R be the probability that the two gametes possessed by an individual are unlike by descent regarding all the loci of set R. Like the inbreeding function, ϕ_R, the quantity π_R can be given as a function of the gametic frequencies belonging to set R, the type of that function depending on the inbreeding system employed. So the term *panmictic function* may be applied to designate the probability π. The panmictic function can be used for constructing recurrence relations for such inbreeding systems as selfing, full-sib mating, and parent-offspring mating. This subject, however, will not be discussed here.

Both the panmictic function and the inbreeding function may be regarded as special cases of a more general quantity, i.e., the probability that the two gametes of an individual are homozygous by descent with respect to a given set of loci, Q, and heterozygous by descent as to a complementary set of loci, $\overline{Q}$, where $(Q + \overline{Q}) = R$. We shall denote this probability by $\xi_{R(Q)}$. Reference to specific sets of loci will be made in the usual way. For example, $\xi_{ijkl(k)}$ denotes the probability that the genes possessed by an individual are identical by descent at locus k, and unlike by descent at loci i, j, l. The case $\overline{Q} = R$ may be denoted by brackets being empty, such as in $\xi_{ijkl()}$.

Concerning a given set of r loci there are 2^r probabilities $\xi_{R(Q)}$ which add to unity. As to the special cases mentioned above, we have the identities, $\pi_R = \xi_{R()}$, and, $\phi_R = \xi_{R(R)}$. Furthermore, each value of the function of inbreeding which refers to a subset of set R is expressible as the sum of appropriate probabilities $\xi_{R(Q)}$. For example, there are such obvious relations as

$$\phi_{ij} = \xi_{ijk(ij)} + \xi_{ijk(ijk)},$$
$$\phi_i = \xi_{ijk(i)} + \xi_{ijk(ij)} + \xi_{ijk(ik)} + \xi_{ijk(ijk)},$$

and so on. Appropriate sets of such expressions of functions of inbreeding, or similar expressions of panmictic functions, can be solved for the respective probabilities $\xi_{R(Q)}$. Table 3 specifies the resulting relations for the three-loci case. General formulations are facilitated by extending a previous convention, *viz.* that $\pi_R = \phi_R = 1$ in the case $r = 0$. Then we may write,

$$\xi_{R(Q)} = \sum_{H \subset Q} (-1)^h \pi_{(H+\overline{Q})} = \sum_{G \subset \overline{Q}} (-1)^g \phi_{(G+Q)}. \qquad (13)$$

As π_R and ϕ_R are special cases of $\xi_{R(Q)}$, formula (13) also furnishes the general relations between panmictic function and inbreeding function.

$$\pi_R = \sum_{Q \subset R} (-1)^q \phi_Q, \text{ and, } \phi_R = \sum_{Q \subset R} (-1)^q \pi_Q. \tag{14}$$

It is only in the case $r = 1$ that the formulae (14) reduce to the simple relation $\pi_R = 1 - \phi_R$, which is equivalent to the definition of the panmictic index, *viz.* $P = 1 - F$.

TABLE 3

Relations between the probabilities ξ, π, and ϕ, for three loci involved

$$\begin{aligned}
\xi_{ijk()} &= \pi_{ijk} = 1 - \phi_i - \phi_j - \phi_k + \phi_{ij} + \phi_{ik} + \phi_{jk} - \phi_{ijk} \\
\xi_{ijk(i)} &= \pi_{jk} - \pi_{ijk} = \phi_i - \phi_{ij} - \phi_{ik} + \phi_{ijk} \\
\xi_{ijk(j)} &= \pi_{ik} - \pi_{ijk} = \phi_j - \phi_{ij} - \phi_{jk} + \phi_{ijk} \\
\xi_{ijk(k)} &= \pi_{ij} - \pi_{ijk} = \phi_k - \phi_{ik} - \phi_{jk} + \phi_{ijk} \\
\xi_{ijk(ij)} &= \pi_k - \pi_{ik} - \pi_{jk} + \pi_{ijk} = \phi_{ij} - \phi_{ijk} \\
\xi_{ijk(ik)} &= \pi_j - \pi_{ij} - \pi_{jk} + \pi_{ijk} = \phi_{ik} - \phi_{ijk} \\
\xi_{ijk(jk)} &= \pi_i - \pi_{ij} - \pi_{ik} + \pi_{ijk} = \phi_{jk} - \phi_{ijk} \\
\xi_{ijk(ijk)} &= 1 - \pi_i - \pi_j - \pi_k + \pi_{ij} + \pi_{ik} + \pi_{jk} - \pi_{ijk} = \phi_{ijk}
\end{aligned}$$

The number of loci homozygous by descent: The category of probabilities $\xi_{R(Q)}$ introduced previously turns out to be useful at least regarding one problem of immediate interest, i.e. the derivation of the variance of the number of loci homozygous by descent, allowing for arbitrary linkages. KEMPTHORNE (1957) mentioned this problem as being not yet generally solved.

Denote by n the total number of loci considered, and by m the number of loci homozygous by descent after a certain system of inbreeding being employed. As is clear from (13), the distribution of the variable m can be described in terms of appropriate values of either the inbreeding function or the panmictic function. Fortunately, the expression of the rth cumulant of that distribution does not involve those values of the inbreeding function (or panmictic function, respectively) that refer to sets of more than r loci at a time. Particularly, the distribution of m has the mean,

$$\overline{m} = \phi_i n,$$

and the variance,

$$\sigma_m^2 = \phi_i n(1 - \phi_i n) + 2\Sigma\phi_{ij}. \tag{15}$$

Here as well as in the following paragraph, Σ means summation over all the pairs of loci which are distinct disregarding order, i.e. $\frac{1}{2}n(n-1)$ items.

We shall exemplify the use of formulae (15) by means of the inbreeding pedigrees considered previously (Figures 1–3). All we have to do is to insert the respective expressions of ϕ_i and ϕ_{ij} reached in (8), (10), and (12) into formulae (15). Of course, the mean number of loci homozygous by descent will be the same for the three pedigrees, *viz.* $\overline{m} = \phi_i n = \frac{1}{4}n$. As to the variances, we get for inbreeding by mating full sibs,

$$\begin{aligned}
\sigma_m^2 &= \tfrac{1}{4}n(1 - \tfrac{1}{4}n) + 2\Sigma\tfrac{1}{16}(1 + \tfrac{3}{2}\lambda_{ij}^2 + \lambda_{ij}^3 + \tfrac{1}{2}\lambda_{ij}^4) \\
&= \tfrac{3}{16}n + \tfrac{1}{16}\Sigma(3\lambda_{ij}^2 + 2\lambda_{ij}^3 + \lambda_{ij}^4).
\end{aligned} \tag{16}$$

Similarly, we find for mating parent to offspring,

$$\sigma_m^2 = \tfrac{3}{16}n + \tfrac{1}{16}\Sigma(2\lambda_{ij} + 2\lambda_{ij}^2 + 2\lambda_{ij}^3), \tag{17}$$

and for mating two sibs one of which has arisen from selfing,

$$\sigma_m^2 = \tfrac{3}{16}n + \tfrac{1}{16}\sum(2\lambda_{ij} + \lambda_{ij}^2 + 2\lambda_{ij}^3 + \lambda_{ij}^4). \quad (18)$$

In all cases the expressions of the variances consist of two parts, one of which is equivalent to $nF(1-F)$, while the other one gives the whole contribution due to linkage. The inbreeding pedigree referred to in (18) is again found to be intermediate between the two other pedigrees as to the effect of linkage. This result, however, is no longer confined to the value of ϕ_{ij} but becomes now evident to concern the whole of the loci involved, *viz.* by affecting the shape of the distribution of the number of loci homozygous by descent.

As regards the genetic significance of the quantity σ_m^2, we only point to the fact that σ_m^2 is the error variance to be attached to the mean, $\overline{m} = Fn$, if the latter quantity is taken as an estimate of the true number of loci homozygous by descent in a particular member of the inbred population. Thus, the higher the value of σ_m^2, the less reliable such a prediction made from the pedigree. In view of this, it seems to be desirable to use either the variance σ_m^2 or the value of ϕ_{ij}, from which the former is deduced, as a supplement to the coefficient of inbreeding, F, for describing the effect of a given system of mating on the homozygosity status of resulting individuals.

Here we have an example of the practical utility of the concept of the function of inbreeding. Other applications of this concept will probably be displayed by further investigations. The usefulness of the function of inbreeding for the interpretation of covariances between relatives is considered elsewhere (SCHNELL 1962).

SUMMARY

New classes of recombination values are defined by means of which the frequencies of modes of gamete formation can be given as linear expressions of recombination values up to any number of loci. The expressions become completely orthogonal after replacing recombination values by a complementary series of parameters, which are named *linkage values*.

Gametic frequencies as well as linkage values are utilized for expressing a quantity, named *function of inbreeding*, which generalizes WRIGHT's coefficient of inbreeding. The function of inbreeding is defined to be the probability that the two gametes of an individual are identical by descent regarding a given set of loci. Similarly, a *panmictic function* is defined. Both the inbreeding function and the panmictic function are special cases of a more general quantity, i.e. the probability that the two gametes of an individual are homozygous by descent concerning a given subset of the loci considered, while being heterozygous by descent as to the remaining subset of those loci.

The defined categories of probabilities are of use in such problems as deriving the cumulants of the distribution of the number of loci homozygous by descent. The variance of that distribution is given in general terms as well as for a few examples of inbreeding pedigrees.

ADDENDUM

After completing this article, the present writer came to know that in a recent paper (Heredity 15: 153–159) R. MORLEY JONES has already extended the linear relationship between gametic frequencies and recombination values up to any number of loci. Yet the additional parameters he defines for that purpose are not identical with the new classes of recombination values as given in the present article.

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SOMATIC FLOWER-COLOR VARIATIONS AND MORPHOLOGICAL CHANGES INDUCED BY C^{14} IN THE SNAPDRAGON, ANTIRRHINUM MAJUS[1]

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A STUDY of the genetic effects of C^{14} uniformly distributed in the tissue of growing plants is of special interest because of three possible processes by which C^{14} so incorporated could be mutagenic.[3] Mutagenic action might result not only from the beta radiation from C^{14} decay but also from the recoil nucleus and its transformation into a nitrogen atom (BRENT 1954).

Increases in atmospheric C^{14} have most recently been noted by BROECKER and WALTON (1959), HAGEMAN, GRAY, MACHTA and TURKEVICH (1959) and WILLIS (1960). The possible mutagenic effects of an increase in atmospheric C^{14} have been considered by TOTTER, ZELLE and HOLLISTER (1958) and PAULING (1958). Recent literature concerning transmutation has been reviewed by POWERS (1956, 1957), who has considered it as a factor which slightly enhances the genetic effects of incorporated P^{32} or other radioisotopes beyond those resulting from equivalent beta irradiation from sources outside of the critical cellular molecules.

Most studies in the field of radiation mutagenesis have dealt with single, high-intensity irradiation of various organisms. Until recently it has been assumed that low-intensity irradiation is equally efficient in the production of mutations; however, recent work by RUSSELL, RUSSELL and KELLY (1958) demonstrated that chronic gamma radiation was significantly less effective than acute X-rays in inducing specific gene mutations. Radioisotope incorporation into organisms provides a means for studying effects of irradiation in which the dose rate is reduced, and effects of atomic recoil and transmutation are also introduced.

In the present experiments, snapdragon plants were exposed to internal irradiation over the major part of their life cycle by growing them in an atmosphere containing CO_2 enriched with C^{14}. Thus, the plants were randomly labeled

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[3] Throughout this paper, the term "mutation" is used in its broadest sense, and includes any genetic change which is implied by a detectable deviation from normal phenotypic expression.

with C^{14} and exposed to relatively uniform dose rates. By varying the C^{14} concentration of the ambient air in different treatments, the mutagenic capacity of various tissue concentrations of the isotope was studied. Observations were made on the growth habit of the plants and on the incidence of somatic mutations in flowers heterozygous for genes affecting flower color.

MATERIALS AND METHODS

Plant material: The common greenhouse snapdragon, *Antirrhinum majus*, was chosen as the experimental plant material, and somatic change in flower color was used as the criterion of genetic change. The genetics of flower color in the snapdragon has been well established by STUBBE (1941) and HANEY (1954), and previous work (SPARROW, DENEGRE and HANEY 1952; SPARROW and POND 1956; and CUANY, SPARROW and POND 1958) indicates that relatively low doses of chronic ionizing radiation affect the somatic mutation rate of the flower color genes.

Experiments to measure somatic mutation frequency were carried out in a purple-flowered F_1 hybrid (Windmillers Lilac × Yuletide) heterozygous with respect to four genes conditioning white, ivory, and lavender flower colors and intensity of flower color. A lavender variety (Windmillers Lilac) was used to study morphological effects.

Culture techniques: Plants were grown in atmospheres in which the CO_2 was enriched with C^{14} in the biosynthesis chambers (SCULLY and CHORNEY 1949; SCULLY *et al.* 1955) at Argonne National Laboratory. Each chamber is a closed system in which plants are grown by nutriculture methods and in which the concentrations of CO_2 and C^{14}, as well as temperature, humidity, and lighting, are controlled and recorded. Temperature in the chambers was maintained at 65±2°F and humidity at 55±5 percent. The day length was extended to 16 hours by artificial light. Nutrient solutions were prepared essentially according to FLINT and ASEN (1953) and were pumped once every other day from nutrient solution tanks into the gravel substrate. Control plants, not exposed to $C^{14}O_2$, were grown under similar conditions but in the open greenhouse where the temperature was maintained between 60°F and 70°F except for a few days during the summer.

C^{14} concentration and specific activity of CO_2 were controlled by generation and release of suitable proportions of labeled and unlabeled CO_2 into the chambers. The concentration of CO_2 was maintained between 0.09 percent and 0.01 percent except for brief periods when the chambers were opened. During the operation of the chambers, particularly during the early portion of a culture period, the specific activity of the air phase shifted constantly downward from the level specified for each treatment. The specific activity was adjusted to 20 percent above the specified level and readjusted upward when it fell to 20 percent below the specified level.

Since only three chambers were available at any time, a first experiment begun in January maintained plants at 4, 25, and 150 μc per gram carbon and a second begun in May utilized specific activities of 150 and 900 μc per gram. Ex-

periment 1 was terminated in 62 days and Experiment 2 in 77 days. Forty-five plants of the F_1 hybrid were introduced into each dosage group as seedlings having about ten macroscopic leaves and an average dry weight of 60 mg. Each group also included ten decapitated plants of Windmillers Lilac (since seedlings were not available) with an average dry weight of 250 mg. Two leaves and two small lateral branches were left at decapitation, and three weeks later a lateral branch was removed from each.

Radiation dosage: Radiation dosages were calculated according to MARINELLI's formula (MARINELLI, QUIMBY and HINE 1948) which gives, on the basis of concentration of isotope, the radiation dose rate in an infinitely thick sample of tissue. This formula may be expressed as Dose = 2.7 C rep per day when C is the concentration of C^{14} in μc per gram of fresh tissue; one rep (roentgen equivalent physical) is equivalent to the absorption of 93 ergs of energy per gram of tissue. Using the measured ratios of dry weight to fresh weight and the activities measured in dry samples, dose rates were then calculated for representative samples. The samples were weighed wet, dried at 80°C, reweighed, ground to pass through a 30-mesh screen, redried, and counted at infinite thickness (about 140 mg per cm^2) in aluminum planchets. Triplicate determinations of duplicate samples were made using a thin-window Geiger-Muller tube. Samples taken at the several levels were assayed for total carbon and C^{14} by DR. ALEXANDER VAN DYKEN of Argonne National Laboratory and used as standards.

Estimates of radiation dosage represent maximum radiation dosage. The minimum radiation dosage at the periphery of the tissue will then be one half of the maximum plus a small increment due to C^{14} in the ambient air.

EXPERIMENTAL RESULTS

Radiation dosages: The approximate radiation dosages, in reps, received by the plants in this study were determined in order that general comparisons might be made between the effects of incorporated radiocarbon and the effects of irradiation from external sources. The dosages shown in Table 1 were calculated from ratios of dry weight to fresh weight and determinations of absolute activity in leaf tissues collected during the flowering period. Analyses of leaf and stem tissue

TABLE 1

Radiation dosages calculated from absolute activities and moisture contents determined in leaf tissue from plants exposed continuously to various concentrations of radiocarbon

Specific activity in air phase (μc per gram carbon)	Dry:fresh weight ratio	Absolute activity (μc per gram dry tissue)	Calculated dosage (rep per day)
	Experiment 1		
4	0.134 ± 0.001	1.914 ± 0.006	0.69
25	0.128 ± 0.004	10.17 ± 0.03	3.5
150	0.132 ± 0.001	64.56 ± 0.16	23.0
	Experiment 2		
150	0.158 ± 0.001	67.35 ± 0.26	28.7
900	0.152 ± 0.003	390.5 ± 1.2	160.

samples collected periodically during the culture period indicated that the C^{14} concentrations of the tissues were not at equilibrium with the C^{14} concentrations of the air phase until after three weeks. Also, the moisture content decreased as tissues matured.

Frequency of mutant areas: Major emphasis in this study was placed on observations of somatic changes in flower color in plants of the F_1 hybrid. Recessive mutations could be detected as sectors or spots of a lighter color in the normal background of dark purple petal color. Flowers were collected from plants of the F_1 hybrid and scored for somatic mutations according to the method of SPARROW and POND (1956). A total area of three square centimeters was scored on each flower. White, ivory, pink, and lavender mutations were theoretically possible, but only white and pink were distinguishable. White mutant areas may have included both white and ivory mutations, and pink mutant areas may have included both pink and lavender mutations. Neither the pink nor the white could be attributed to mutations at specific loci. Each light-colored area was scored as one mutation.

The frequencies of mutant areas observed on petals of plants exposed to the various concentrations of radiocarbon are shown in Table 2. The frequency of both pink and white mutant areas increased progressively with increase in radiocarbon concentration. On the average, pink mutant areas were observed 7.5 times as frequently as white. There were no consistent differences between superior and inferior petals with respect to the frequency of mutant areas. The total number of mutant areas per flower (3 square cm) increased from 0.61 in control plants to 28.06 in plants exposed to an air-phase specific activity of 900 μc per gram carbon. The somatic mutation rate observed in plants exposed to an air-phase specific activity of 4 μc per gram carbon was approximately twice the rate in control plants.

The mutant area data from each treatment were compared with the data from

TABLE 2

Frequencies of mutant areas observed on petals of F_1 hybrid snapdragon plants exposed to various concentrations of radiocarbon

Specific activity in air phase (μc per gram carbon)	No. of plants	No. of flowers	Mutant areas per flower (3 square cm)		
			Pink	White	Totals* (pink and white)
		Experiment 1			
Control	44	217	0.46	0.06	0.51 (0.61)
4	24	177	1.10	0.14	1.24
25	24	190	1.31	0.22	1.53
150	24	139	4.58	1.22	5.80 (5.66)
		Experiment 2			
Control	30	186	0.66	0.06	0.73 (0.61)
150	23	146	4.79	0.73	5.53 (5.66)
900	21	93	25.47	2.59	28.06

* The figures in parentheses in this column indicate the frequencies of mutant areas obtained when data from corresponding treatments in the two experiments were combined.

each of the other treatments. The average number of mutant areas per flower for each plant was ranked, and the H test, discussed by KRUSKAL and WALLIS (1952), was applied to the ranked data. Comparisons between the two control groups and between the two groups of plants exposed to an air-phase activity of 150 μc per gram carbon revealed no significant differences in the frequencies of mutant areas between corresponding treatments of Experiment 1 and Experiment 2. Therefore, it was possible to combine the mutation data from corresponding treatments in the two experiments. The averages of the total number of mutant areas per flower for the plants in the control groups and for the plants exposed to an air-phase activity of 150 μc per gram carbon are shown in parentheses in the last column of Table 2. The differences between the control and the 4 μc per gram carbon treatments and the difference between the 4 and 25 μc per gram carbon treatments were significant at the five percent level of probability. The differences in the frequency of mutant areas between treatments in the various other possible comparisons were significant at the one percent level of probability.

Morphological effects: Visible morphological changes were not induced in plants grown in air-phase activities of 4, 25 or 150 μc per gram carbon. The last specific activity induced calculated dose rates of 23.0 and 28.7 rep per day in Experiments 1 and 2, respectively. On the other hand, plants exposed to the 900 μc per gram carbon level exhibited abnormal and reduced branching and flower production, increases in leaf thickness, stem diameter, and plant height, and abnormalities in flowers. In many instances, small vegetative branches instead of flowers were formed on the flowering stalk or peduncle. The most typical flower abnormalities consisted of twisted or buckled petals, cleft petals, and degenerate or reduced stigma, style, and ovary. In comparison with the F_1 hybrid, the Windmillers Lilac plants were more severely affected by the radioactivity, produced fewer lateral branches, and did not flower during the time they were exposed to the radiocarbon dioxide. Dichotomous branching of the main stem was observed in three of the eight Windmillers Lilac plants exposed to the 900 μc per gram carbon activity level.

The average lengths of stems, measured in centimeters from the cotyledonary node to the base of the lowermost flower, are shown in Table 3. In Experiment 1, there was no consistent difference in average length of stem among the different radioactivity levels. In Experiment 2, the plants exposed to an air-phase activity of 900 μc per gram carbon had a greater average length of stem than either control plants or plants exposed to the 150 μc per gram carbon activity. The average height from the cotyledonary node to the apex of the main stem in plants exposed to the 150 and 900 μc per gram carbon activities in Experiment 2 are shown in parentheses in Table 3. These data show clearly that plants were taller at the higher activity than at the lower activity.

DISCUSSION

The values representing the effectiveness of different dosages in production of mutant areas presented in Table 4 were calculated for use as indices of the relative

TABLE 3

Mean length of stem from the cotyledonary node to the base of the lowermost flowers on the main stems of plants of Windmillers Lilac and the F_1 hybrid exposed to various concentrations of radiocarbon*

Specific activity in air phase (μc per gram carbon)	Windmillers Lilac Plants (no.)	Windmillers Lilac Stem length (cm)	F_1 hybrid Plants (no.)	F_1 hybrid Stem length (cm)
		Experiment 1		
Control	10	29.1 ± 0.9	44	32.3 ± 0.6
4	8	32.0 ± 1.0	23	41.0 ± 0.6
25	8	31.7 ± 1.3	24	45.1 ± 0.7
150	6	33.3 ± 0.5	24	43.2 ± 0.6
		Experiment 2		
Control	16	42.6 ± 1.5	24	40.5 ± 0.5
150	8	34.8 ± 0.9	23	32.9 ± 0.6
		(46.3 ± 1.6)		(44.5 ± 1.2)
900	8	.....	24	46.5 ± 0.6
		(83.8 ± 8.6)		(77.8 ± 3.0)

* The figures in parentheses represent the mean length of stem from the cotyledonary node to the apex of the main stem.

TABLE 4

Relative effectiveness of different dosages of C^{14} in production of mutant areas in snapdragon flowers

Air phase specific activity (μc per gram carbon)	Calculated dose rate (rep per day)	Mutant areas per flower per μc per g carbon*	Mutant areas per flower per rep per day*
	Experiment 1		
4	0.69	0.18	1.06
25	3.5	0.04	0.29
150	23.0	0.04	0.23
	Experiment 2		
150	28.7	0.03	0.17
900	160.0	0.03	0.17

* Calculated by subtracting the control rate from the experimental and dividing the remainder by either the air-phase specific activity or the daily dose rate.

efficiency of radiocarbon at different concentrations. The values representing mutant areas per flower per μc per gram carbon and mutant areas per flower per rep per day were calculated by subtracting the control rate from the experimental and dividing the remainder by the air-phase specific activity (μc per gram carbon) and the calculated daily dose rate (rep per day), respectively. With the exception of the 4 μc per gram carbon treatment, the data indicate that the efficiency of the radiocarbon treatments was approximately 0.03 mutant areas per flower per μc per gram carbon. In the 4 μc per gram carbon treatment, the calculated efficiency was approximately five times as high as the efficiency in the other treatments. Indices of efficiency would be constant for all treatments if the dose-response relationship were linear. The amount of data from treatments involving low C^{14} concentrations is limited in this study and precludes a definite

conclusion that the mutant area response departs from linearity at low air-phase specific activities. SPARROW and POND (1956) observed similar deviations from a linear dose-response relationship at low dose rates of gamma radiation in certain of their experiments involving mutant areas in snapdragon flowers. However, recent tests (CUANY *et al.* 1958) have not shown higher mutagenic efficiency for lower dose rates of gamma and X-rays.

As shown in Table 2, the dosage which doubled the spontaneous rate was approximately 4 μc per gram carbon or 0.69 rep per day. If the number of somatic mutations per μc/gram carbon were indeed 0.03 or 0.04 instead of 0.18 (Table 4), the dosage required to double the mutation rate would be 15 or 20 μc per gram carbon instead of four.

The values in the last column of Table 4 were calculated primarily for the purpose of comparing the effectiveness of the radiocarbon treatments with the effectiveness of chronic gamma irradiation. With respect to radiocarbon treatments, the frequency of mutant areas per flower per rep per day ranged from 0.17 at 160 rep per day to 1.06 at 0.69 rep per day. Both extremes of this range are within the range of 0.01 to 2.95 mutations per daily r unit reported by SPARROW and POND (1956) for gamma-ray dose rates of 272r per day (July, 1955) and 2.2r per day (July, 1953), respectively. A large amount of seasonal and yearly variability of mutation rates occurred in the gamma-radiation studies, and differences in effectiveness between the two types of treatment are not consistent from year to year or from season to season. Facilities for conducting chronic gamma radiation and radiocarbon treatments under similar environmental conditions were not available, and accordingly data suitable for direct comparison of the two types of treatments could not be obtained at this time. Critical evidence regarding differences in effectiveness is lacking, but the comparison of ranges indicates that the two types of treatment may be equally effective.

Morphological effects such as reduced lateral branching and flower production, increased leaf thickness, stem diameter, and plant height, and dichotomous branching were observed in snapdragon plants exposed to a dosage of 160 rep per day (air-phase activity of 900 μc per gram carbon). Similar morphological effects were observed in snapdragon plants exposed to chronic gamma irradiation (GUNCKEL 1957; GUNCKEL and SPARROW 1954; SPARROW and SINGLETON 1953), based on 20-hour daily irradiation periods. With the exception of increase in stem height and diameter, the effects from chronic gamma irradiation were not observed at dose rates below 240r per day. Thus, on the basis of equivalent amounts of ionization, it appears that incorporated radiocarbon may be slightly more effective than gamma rays in producing certain morphological changes.

GUNCKEL and SPARROW (1954) first noted increases in stem height and diameter in snapdragon plants exposed to gamma radiation (240 to 350r per day). Later studies reported by SPARROW and GUNCKEL (1955) noted that these responses were induced at rates above 125r per day and reached a maximum at 230r of gamma radiation. Accordingly, such types of plant growth stimulation are promoted at comparable dose rates of beta (160 rep) and gamma radiation. Although considerable controversy has long existed regarding the capacity of

ionizing radiations to stimulate plant growth, the above data for snapdragon supports SAX's (1955) opinion that there is increasing critical evidence of such growth promotion.

In the present study the promotion of elongation of the main stem axis of snapdragon was associated with the suppression of normal, active lateral bud growth in the region below the terminal inflorescence. The increase in stem elongation occurred both in the region below and within the terminal, racemose inflorescence. The major part of the increase in the total stem length was associated with a prolongation of the differentiation of vegetative main axis nodes or delay in initiation of nodes which eventually bore the macroscopic mature flowers of the inflorescence. This pattern of growth stimulation occurred at a dose rate that was 230 times higher than that at which a doubling of the spontaneous, somatic mutation rate occurred in the petals.

Mutagenic effects of atomic transmutation were indicated by experiments with S^{35} in Neurospora (HUNGATE and MANNELL 1952), P^{32} in Drosophila (ARNASON, IRWIN and SPINKS 1951), P^{32} in *Escherichia coli* (RUBIN 1950), P^{32} in bacteriophage (HERSHEY, KAMEN, KENNEDY and GEST 1951), and P^{32} in *Paramecium aurelia* (POWERS 1948). Genetic effects of atomic transmutation were not evident in work by KING (1953) with P^{32} in Drosophila or in that by POWERS and SHEFNER (1951) with H^3 in *Paramecium aurelia*. STENT (1953) and CASTOGNOLI and GRAZIOSI (1954) found that the rate of inactivation of bacteriophage by assimilated P^{32} was temperature-dependent. STENT suggested that recoil of the S^{32} atoms might be involved. In order to distinguish between the effects of transmutation and radiation, it is necessary that the radiation dosage be not completely dependent upon the concentration of the assimilated radioisotope. Since addition of supplemental external radiation was not provided in this experiment, differentiation between the effects of transmutation and radiation was not possible. In fact STRAUSS (1958) suggested that detection of a transmutation effect of C^{14} in uniformly labeled material may be impossible. This author noted that the elements most likely used in transmutation studies with the exception of P^{32} do not have sufficiently high, maximum recoil energies to induce orbital electron loss through nuclear acceleration associated with decay. This evaluation applies to H^3, C^{14}, P^{33}, S^{35}, and Ca^{45}. KAUDEWITZ, VIELMETTER and FRIEDRICHFRESKA (1958), in studies using *Escherichia coli* B/n, clearly demonstrated that the radioactive decay of P^{32} atoms which are incorporated into the living cells, induce mutations which could not be ascribed to the associated beta rays.

Addition of supplemental external radiation as a control for the evaluation of the specific mutagenic effect possibly ascribable to transmutation was not possible in this experiment. The fact that the mutagenic effectiveness of C^{14} was similar to that of chronic gamma irradiation indicates that transmutation was not an important factor in causing mutations in the snapdragon. Few biological data are available for evaluation of the genetical effects of transmutation of this isotope, at least for tissues which are uniformly labeled. In man, TOTTER *et al.* (1958) estimated that the transmutation of C^{14} could lead to about the same number of mutations as the radiation from C^{14}.

It was assumed that the light areas observed in the snapdragon flowers were the result of heritable changes in the somatic tissue. The distinctness of the boundaries of the light areas indicated that the spots were the result of changes affecting pigment production which were heritable in somatic cell division during petal development. Since it was not possible in this study to subject the somatic mutations to genetic or cytogenetic tests, nothing is known about the nature of these changes. A few of the more obvious possibilities might be listed as follows: (1) recessive gene mutations, (2) deletions, (3) position effects associated with chromosomal rearrangements, (4) inherited cytoplasmic changes, and (5) radiation-induced somatic crossing over and subsequent mitotic segregation.

It is pertinent to note that 4 μc of C^{14} per gram of tissue carbon induced a doubling of the spontaneous, somatic mutation rate of color in snapdragon flowers. This concentration is approximately 5×10^5 times as high as the natural levels determined first by LIBBY (1952), by BROECKER and WALTON (1959) for 19th century wood, and for the highest current activity level reported by WILLIS (1960) for oat seed cultured in 1959.

SUMMARY

1. The genetic and morphological effects of radiocarbon when incorporated into plants of the common snapdragon, *Antirrhinum majus*, were investigated. Plants of an F_1 hybrid heterozygous for flower color and plants of the variety Windmillers Lilac were exposed to atmospheres containing carbon dioxide enriched with $C^{14}O_2$. The estimated radiation dosages were 0.69, 3.5, 25.8, and 160 rep per day for plants grown in air-phase specific activities of 4, 25, 150, and 900 μc per gram carbon, respectively.

2. The frequency of somatic mutations increased from 0.61 mutant areas per flower in controls, to 1.24, 1.53, 5.66, and 28.06 mutant areas per flower at air-phase activities of 4, 25, 150, and 900 μc per gram carbon, respectively. The relationship between air-phase specific activity and frequency of mutant areas appeared to be linear at air-phase specific activities from 25 to 900 μc per gram carbon; the efficiency of radiocarbon treatment was greatest at 4 μc per gram carbon. Only two phenotypic classes of mutant areas, *viz.*, pink and white, could be classified accurately. Neither the pink nor the white areas could be attributed to mutation at specific loci. On the average, pink mutations were observed 7.5 times as frequently as white.

3. No morphological changes in plants of the F_1 hybrid and Windmillers Lilac were observed at the three lower air-phase specific activities. Plants exposed to the 900 μc per gram carbon air-phase activity exhibited reduced lateral branching and flower production, increases in leaf thickness, stem diameter, and plant height, abnormalities in flowers, and dichotomous branching of the main stem.

4. Comparisons with the results of other workers indicate that radiocarbon treatment was slightly more effective than equivalent amounts of ionization from chronic gamma irradiation in producing certain morphological changes. Radio-

carbon and chronic gamma irradiation were not shown to differ in effect in the induction of somatic flower color mutations in snapdragons.

5. The lowest concentration of radiocarbon which was effective in doubling the spontaneous somatic flower color mutation rate of snapdragon is 5×10^5 times as high as the highest naturally occurring concentration as reported in 1959.

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PSEUDOTUMORS AND FITNESS IN DROSOPHILA MELANOGASTER AND DROSOPHILA SIMULANS

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MELANOTIC tumors ("pseudotumors") in Drosophila have been found to be very widespread in almost all laboratory stocks of *D. melanogaster* (BURDETTE and OLIVIER 1952; BARIGOZZI and DI PASQUALE 1956) and in several natural populations of different species (BRNCIC and KOREF 1953; MAKINO and KANEHISA 1954; DI PASQUALE and KOREF-SANTIBAÑEZ 1959).

Although this trait is very variable in its expression, and most probably in its genesis, the following are some of the characteristics common to all "pseudotumors": (a) In *D. melanogaster* their presence is due fundamentally to main genes localized on the second chromosome and to several modifiers on the others (BURDETTE and OLIVIER 1952; BARIGOZZI and DI PASQUALE 1956). (b) Their genetic determination is strongly heterogeneous, for in different stocks with a similar phenotype, nonallelic genes are involved (BARIGOZZI and DI PASQUALE 1956). (c) Their spontaneous incidence is low but may sometimes be increased by inbreeding (MAKINO and KANEHISA 1951). (d) Their incidence is modified by changes in the environment (HARNLY, FRIEDMAN and GLASSMAN 1951; HERSKOWITZ and BURDETTE 1951).

KOREF (1956) has postulated that these pseudotumors could be considered, owing to all these general characteristics, as *phenodeviants* (LERNER 1953).

As a complement to the above hypothesis, DI PASQUALE and KOREF-SANTIBAÑEZ (1959) found that in natural populations of *D. melanogaster* and *D. simulans* pseudotumors were observed in high frequency in the individuals collected in nature. Moreover, in the descendants of single wild females, after 12 generations under laboratory conditions, without marked inbreeding or selection for the pseudotumoral trait, this trait was not eliminated. On the contrary, in certain cases the incidence of pseudotumors tended to increase. Another interesting fact was the marked genetic heterogeneity among the different lines, each of which originated from a single female, which resulted in very low tumor percentage following crossing of individuals of different tumor lines (DI PASQUALE and KOREF-SANTIBAÑEZ 1959). All this led the authors to postulate that these "pseudotumors" could in some way contribute to a higher fitness of their carriers which would therefore favor the maintenance of the trait in the populations.

The authors think that observations on the pseudotumors incidence in descendants from individuals captured in nature and maintained under different systems of breeding in order to allow them to reach different grades of homozy-

gosis may help to clarify the nature of the trait. In fact, if increasing in homozygosis causes a higher tumor incidence, this might support the hypothesis that pseudotumors are "phenodeviants."

On the other hand, it seems interesting to inquire if in pseudotumor individuals or lines some of the most important components of fitness have higher values than in tumorless ones. In the affirmative case, one could conclude that the adaptative value of the tumoral genotype plays a role in maintaining the high incidence of the trait in the populations.

MATERIALS AND METHODS

Two main studies were here carried out in the two sibling species, *D. melanogaster* and *D. simulans*.

1. An analysis of pseudotumor incidence was made in several lines, each of which was derived from a single female captured in nature (at Aspra) and kept under different levels of inbreeding.
   A. Four females were crossed with four males of the same generation—36 lines of *D. melanogaster* and 94 lines of *D. simulans* ("medium inbreeding").
   B. Strict individual brother-sister mating was employed in 30 lines of *D. melanogaster* and 95 of *D. simulans* ("high inbreeding").
2. An investigation was made of some of the main components of fitness (BUZZATI-TRAVERSO 1953) such as fecundity, viability, sterility and rate of development. These elements were compared in the following types of stocks or lines.
   A. Laboratory stocks with pseudotumors and laboratory stocks without pseudotumors. *D. melanogaster:* Varese (zero percent *tu*); *tu* B3 (100 percent *tu*); *D. simulans:* Aspra (zero percent *tu*); *tu* B1 (100 percent *tu*).
   B. Lines originated from wild females, both tumoral and nontumoral.

   D. melanogaster: line 52, maintained by medium inbreeding, with an increasing tumor incidence (from 7.6 percent in F_1 to 60.7 percent in F_{24}); line 14, maintained by medium inbreeding, with a low and stable pseudotumor incidence (less than one percent); line 19, highly inbred for three generations, with a low pseudotumor incidence, which frequently fell to zero percent; the same line 19, highly inbred for seven generations; line 8, highly inbred for three generations, with a low and stable pseudotumor incidence (1.2 percent), which is never reduced to zero; line 26, highly inbred for seven generations, with a low and stable incidence (5.7 percent).

   D. simulans: line 37, mediumly inbred, with an increasing pseudotumor incidence (from 5.3 to 81.7 percent); line 8, mediumly inbred, with zero percent pseudotumors in all generations; line 68, highly inbred for three generations, with a low and stable pseudotumor incidence (5.0 percent); the same line 68, highly inbred for seven generations; line 110, highly inbred for three generations, with a very low pseudotumor incidence (less than one percent); line 48, highly inbred for three generations, with a stable pseudotumor incidence (20.0 percent); line 107, highly inbred for seven generations, with in-

creasing pseudotumor incidence (from 5.2 to 70.8 percent); line 115, highly inbred for seven generations, with a stable low incidence (less than one percent).

C. Within lines with high pseudotumor incidence, individuals carrying the trait were compared with individuals free from tumors. The following were the methods used for analyzing the above mentioned components of fitness. All experiments were made at a constant temperature of 24°C.

Fecundity: Daily egg production per female of 20 to 30 females of each stock or line was observed over a period of 20 days. For this purpose, 12-hour old males and females were aged separately for 24 hours and paired individually. Twenty-four hours later, in order to normalize oviposition, each couple was placed in an empty vial (3 × 8 cm), into which was introduced a plastic spoon with the usual Drosophila food medium, blackened with carbon, and touched with a suspension of fresh yeast. The spoons were renewed every 24 hours, and the eggs laid by each female were counted under a binocular microscope.

Viability: The analysis was based on the estimation of the percentage of adults emerged from a known number of eggs. The eggs obtained in the study of fecundity were used. Twenty-five eggs were put into each vial (five eggs per female), and emerged adults were counted. With this method, samples were obtained from females of equal age, and viability was controlled in relation to the age of female.

Sterility: One female was placed into each culture vial together with two males. Vials in which larvae appeared were considered fertile, and where no development was apparent the flies were transferred every four days to new cultures. If after 20 days no development occurred, the females were considered sterile.

Rate of development: Males and females collected within a period of 12 hours were allowed to age separately for 24 hours, mated, and left together for 48 hours. Groups of 20 pairs were placed in one fourth liter milk bottles, whose mouth was covered with a watch glass containing darkened Drosophila food painted with a suspension of fresh yeast. The flies were allowed to oviposit on this food over periods of four hours, after which the eggs were collected and placed 50 per 12 × 3 cm food vial. Two egg samples were taken each day (eggs laid between 9 A.M. and 1 P.M., and between 1 P.M. and 5 P.M.), over a period of six days. Each group of 20 couples was replicated once. The emergence of imagoes was scored every four hours (4–8–12 A.M., 4–8–12 P.M.); since the first controls showed very few flies emerged at 4 A.M. or 12 P.M., these counts were eliminated. The rate of development, expressed in hours, is therefore ± two hours from the period of egg laying to the emergence of adults.

RESULTS

Pseudotumor incidence in lines with medium and high inbreeding: In 66 lines of *D. melanogaster* and 189 of *D. simulans*, the pseudotumor incidence shows a varied behavior in the course of the generations under laboratory conditions (Table 1). Fluctuations of less than 20 percent in absolute incidence have not

TABLE 1

Classification of lines under different degrees of inbreeding, as respects behavior of pseudotumoral incidence under laboratory conditions

		Stable	Increasing	Decreasing	Irregular	Generations	No. lines	No. individuals
D. mel.	Medium inbreeding	32	1	1	2	14	36	45203
		88,9%	2,8%	2,8%	5,6%			
	High inbreeding	30	..	..	..	8	30	16532
		100%						
D. sim.	Medium inbreeding	74	8	1	11	14	94	108223
		78,7%	8,5%	1,1%	11,7%			
	High inbreeding	87	2	..	6	8	95	51185
		91,6%	2,1%	..	6,3%			

been considered as being meaningful. This was true for the majority of the lines, studied in some cases for eight generations (if the line did not become extinct before then); these lines are considered "stable." A small number of lines increased their tumor incidence by an absolute amount of over 20 percent; these are classified as "increasing." A few others showed a decrease of their pseudotumor incidence of more than 20 percent; these are the "decreasing" lines. Finally, a few lines, considered as "irregular," showed oscillations of more than 20 percent, but reached, at the end of the study, incidences which do not differ substantially from those in the first generation (Figure 1).

In both species, the behavior is qualitatively very similar, and although there seem to exist quantitative differences (a greater number of irregular and increasing lines in *D. simulans*), this difference is difficult to prove statistically.

It is also difficult to make a statistical comparison of lines maintained by medium and high inbreeding. Nevertheless, Table 1 shows that in *D. simulans*, although inbreeding seems to decrease the number of lines that show increasing or irregular pseudotumor incidence, the stability of most lines is not affected.

In order to know more about the behavior and structure of some stable and unstable lines, the genotypes of two lines of *D. melanogaster* were analyzed. One of these was an "increasing" line (52), and the other a "stable" one (69). An analysis of isogenic lines for certain chromosomal combinations (obtained by means of substitution of chromosomes containing balanced lethals) indicated that: (a) in both lines the trait is recessive; (b) the main factor or factors ("*tu*") responsible for the pseudotumoral trait are apparently limited to the second chromosome, while modifiers are localized on the other chromosomes, with the third chromosome being especially active in line 52.

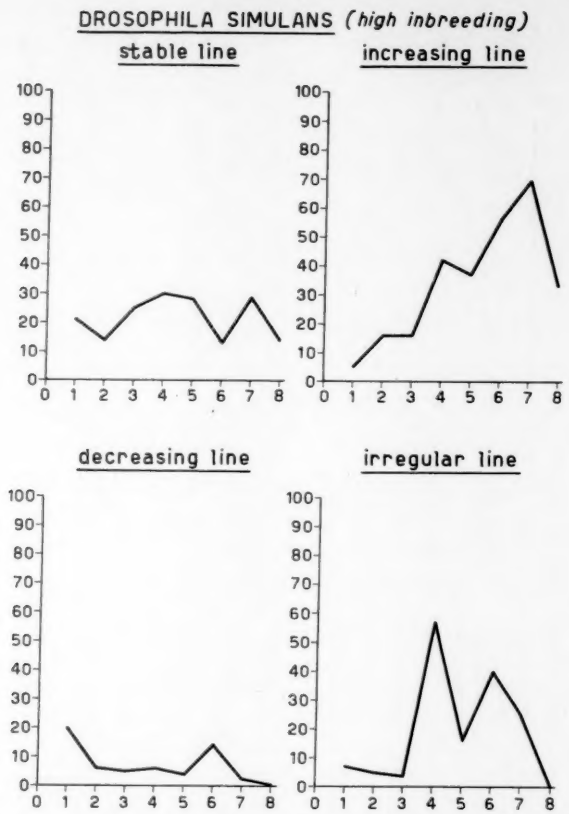

FIGURE 1.—Behavior of the four types of individual lines: stable, increasing, decreasing, irregular. Ordinate: percent of tumors. Abscissa: generations.

Analysis of the components of fitness (Tables 2 and 3)

Fecundity: The mean number of eggs per day per female, and the mean total of eggs per female were recorded. Both these factors are characteristic for each line of each species, although in general *D. melanogaster* had a higher oviposition rate than *D. simulans*. In most cases egg laying remained constant throughout the 20 days. Only in two lines, one of each species (26 of *D. melanogaster* and *tu* B1 of *D. simulans*) was there a tendency towards decrease with age.

In order to find differences in fecundity between tumoral and nontumoral stocks or lines, the data were compared by means of an analysis of variance. Thus, the total number of eggs per fly (20 flies per line) were compared with the corresponding values of 20 flies of the stock used as a control.

TABLE 2

Elements of fitness in D. melanogaster

Line	Percent *tu*	Fecundity: Average no. daily eggs/female	Fecundity: Average total eggs/female	Fecundity: No. females	Viability: Percent reaching adulthood	Viability: No. eggs	Sterility: Percent	Sterility: No. females	Rate of development: Mean no. hours to emergence	Rate of development: No. adults
Varese	0.0	45.3±3.11	828.2	20	87.0±1.23	745	0.0	10	292.9±0.81	231
tu B3	100.0	48.4±2.74	890.7	20	85.8±1.27	760	0.0	10	302.5±1.09	181
52 (1957) ♀ wt ♂ wt*		30.8±5.25	542.7	15	48.7±3.06	267	50.0	8	292.0±1.34	95
52 (1957) ♀ wt ♂ tl†					54.9±2.35	450	14.3	7		
52 (1957) ♀ tl ♂ wt		46.4±3.31	795.1	15	77.0±2.54	274	0.0	5		
52 (1957) ♀ tl ♂ tl					46.8±2.31	466	30.0	10	303.1±2.04	75
52 (1957) Total	60.7	38.6±3.37	668.9	30	55.3±1.30	1457	26.7	30	299.6±1.36	85
14 (1957)	0.0	38.9±3.79	710.7	20	70.7±1.21	1408	10.0	20	301.4±2.30	30
8 (1958) F_3	1.2	36.9±1.61	658.3	20	73.9±1.53	820	10.0	10		
19 (1958) F_3	0.0	35.8±3.90	601.1	19	66.2±1.92	610	0.0	10	268.1±1.01	171
19 (1958) F_7	0.0	40.5±4.15	752.1	20	70.6±1.22	1396	0.0	20	271.2±0.41	729
26 (1958) F_7	5.7	23.8±1.85	316.5	20	16.3±1.33	773	45.0	20	267.3±0.70	229

* wt = with tumor.
† tl = tumorless.

TABLE 3

Elements of fitness in D. simulans

Line	Percent *tu*	Fecundity: Average no. daily eggs/female	Fecundity: Average total eggs/female	Fecundity: No. females	Viability: Percent reaching adulthood	Viability: No. eggs	Viability: Percent	Viability: No. females	Rate of development: Mean no. hours to emergence	Rate of development: No. adults
Aspra	0.0	38.9±1.77	689.4	20	79.0±1.50	735	0.0	10	302.4±0.53	536
tu B1	100.0	20.9±1.44	355.3	20	45.1±2.04	597	30.0	10	288.9±0.51	454
37 (1957) ♀ wt ♂ wt		21.8±3.95	420.5	18	75.3±1.30	1095	16.7	18	285.0±0.72	264
37 (1957) ♀ tl ♂ wt		21.9±3.46	434.4	12	77.1±1.66	641	0.0	8		
37 (1957) ♀ tl ♂ tl					81.6±2.48	245	25.0	4	300.3±1.37	131
37 (1957) Total	81.7	21.8±2.70	426.1	30	76.7±0.95	1981	13.3	30	286.0±0.97	169
8 (1957)	0.0	18.9±1.60	373.3	19	50.2±1.30	1468	15.8	19	300.4±0.82	631
68 (1958) F_3	5.0	34.9±2.48	581.2	20	68.5±1.70	746	0.0	9	263.5±0.58	447
110 (1958) F_3	0.0	30.8±3.19	403.9	19	62.3±2.13	520	10.0	10	266.6±1.46	241
48 (1958) F_3	26.6	30.5±1.81	479.9	19	47.6±1.89	695	30.0	10	262.1±1.09	256
107 (1958) F_7 ♀ wt ♂ wt		15.7±1.58	273.2	14	58.9±2.32	448	28.6	7	265.4±0.54	391
107 (1958) F_7 ♀ wt ♂ tl					68.9±2.16	312	14.3	7		
107 (1958) F_7 ♀ tl ♂ wt		13.4±2.27	243.0	16	65.5±3.12	232	50.0	6		
107 (1958) F_7 ♀ tl ♂ tl					68.4±2.24	566	11.1	9	261.7±0.45	447
107 (1958) F_7 Total	70.8	14.5±1.28	257.2	30	65.3±1.37	1558	24.1	29	262.0±0.62	292
68 (1958) F_7	2.7	36.6±2.69	691.5	20	18.3±1.25	956	40.0	20	257.8±0.48	1054
115 (1958) F_7	0.0	21.2±1.50	404.9	20	55.0±1.27	1541	20.0	20	248.6±0.37	940

In *D. melanogaster*, all the lines and the *tu* B3 stock were compared with the nontumor bearing Varese stock. In line 52, females with tumors were taken separately from nontumoral females. The results of this analysis show no difference in egg laying between the flies from the tumor and the nontumor lines. In line 52, females free from tumors tended to lay more eggs than those with tumors. The low egg production of line 26 in the seventh generation of inbreeding may be attributed to this last fact.

In *D. simulans*, all lines were compared to the nontumor stock, Aspra. In line 37 and 107, egg production by tumor and nontumor females was considered separately. The Aspra stock had a significantly higher egg production than all lines excepting line 68 at the third and the seventh generations of inbreeding. Therefore, another comparison was made between the tumor stock, *tu* B1, and all others. This analysis showed no special tendency of egg production which could be related to the tumor trait. Within the tumor-bearing lines, there was no difference between tumor and nontumor females. Inbreeding does not seem to have affected oviposition in the line 68, although it may be responsible for its reduction in line 107.

Viability: Tables 2 and 3 show the percentage frequency of viability over the total number of eggs considered. Here also the percentage of individuals which reach adult age is characteristic for each stock or line. The possible relationship between age and fertility was calculated by the method of Skory for χ^2; the comparison of the total fertility of all lines was also carried out by the χ^2 method.[1]

In *D. melanogaster*, aging does not seem to influence the viability. Only in F_7 of lines 19 and 26 was there a significant decrease of individuals which emerged from the pupa, the older the parents. This may be attributed to inbreeding.

The comparison with the Varese stock showed no difference with respect to *tu* B3 (both are also the stocks with the highest viability). All other stocks, especially lines 26 and 52, were less viable. In line 52, the viability was lower when both parents were either tumor or nontumor bearing, and higher when one of the parents carried the trait.

In *D. simulans* only three lines, *tu* B1, 110 F_3 and 68 F_7, showed a decrease of the viability with age. The latter line had a very low total viability. Considering the tumoral stocks, only *tu* B1 showed any decrease in viability in relation to the Aspra stock. In all others, the number of adults emerged was slightly greater. Each of the lines with high tumoral incidence behaved differently. In lines 37 and 107, viability was lower when both parents have tumors than when both, or one, did not show the trait.

Summarizing, there is no clear tendency for a correlation of the viability with the tumoral trait. Only when the parents have no tumors does it seem to be slightly higher.

[1] Formula χ^2: $(ad - bc - \frac{n}{2})^2 \times n/(a+b)(a+c)(b+d)(c+d)$ where a,b,c,d, are the frequencies of the four classes considered, and in this case a and c correspond to the adults of two lines, while b and d correspond to the individuals which failed to reach the adult stage; n is $a+b+c+d$.

Sterility: The results are of limited value, as the number of individuals and of lines studied was low.

In *D. melanogaster* there was no difference between the nontumor (Varese) and the tumor (*tu* B3) stock. In line 52, especially when both parents were either tumor or nontumor bearing, the sterility was high. Line 26, at the seventh generation of inbreeding, was also more sterile.

In *D. simulans*, although *tu* B1 is often sterile, there was no relation between this factor and tumor bearing, for in line 37 the highest sterility was found when both parents were either tumor bearing or nontumor bearing. In line 107, on the other hand, the least fertile were the following combinations: both parents tumor bearing, or female without tumors × male with tumors.

Rate of development: Rate of development is expressed as the mean hours which individuals of each line take from oviposition to imago emergence. The data of the different lines were compared by "Student's t." As in the aforementioned components of fitness, this one also shows important individual differences in each line.

In *D. melanogaster*, mean development took from 257.3 to 303.1 hours. Lines captured more recently in nature took less time, while inbreeding did not seem to exert any influence (line 19 in F_3: 268.1 hours, in F_7: 271.2 hours).

The comparison of the rates of development of all lines with the nontumor stock (Varese) shows that *tu* B3 and line 52 took significantly longer (302.9 and 299.6 hours, respectively). Within line 52, development was shorter in descendants parents with tumors (292.0 hours) than in those of nontumor flies (303.1 hours).

In *D. simulans* the mean development time ranged from 248.6 hours to 302.4 hours, the most rapid being also from the more recently captured flies. The comparison with the nontumoral stock (Aspra), shows that *tu* B1 had a shorter development (288.9 hours); the other two lines with intermediate times (line 37: 286.0 hours; line 107: 262.0 hours). Within line 37, development was more rapid in descendants of parents with tumors (285.0 hours) than in those without tumors (300.3 hours). Within line 107, on the other hand, descendants of nontumor flies took less time (261.7 hours) than did those with tumor (265.4 hours).

DISCUSSION AND CONCLUSION

The study of pseudotumors in 66 lines of *D. melanogaster* and 189 lines of *D. simulans*, originated from females captured in nature and maintained for several generations without selection for the tumor trait, shows that different lines have their own particular behavior with respect to pseudotumor incidence. This may mean that the genetic factors responsible for the trait may be different (although in lines 52 and 69 of *D. melanogaster* main genes were found to be recessive and localized in the second chromosome). It may also confirm (KOREF 1955) that the degree of penetrance and expressivity of the *tu* genes depends not only on the same *tu* genes but also on the rest of genotype. Therefore, the natural populations from which the original females were obtained must have been large and genetically very heterogeneous.

As to inbreeding, this does not seem to affect the tumor incidence in the lines here studied. Thus one of the most important elements to support the phenodeviant nature of pseudotumors is lacking. On the other hand, the fact that inbreeding maintains the irregular behavior of pseudotumor incidence in *D. simulans* agrees with the results obtained by BARIGOZZI (1960) in isogenic lines of *D. melanogaster*.

As to the components of fitness, which could clarify the possible adaptative value of the tumoral phenotype, none of those examined gave clear results. Although in line 52 fecundity was decreased, all four components have similar values in the line 110 of *D. simulans* (zero percent *tu*) and in line 37 of the same species (70 percent *tu*). In any case, no general tendency was found to have a higher fitness when pseudotumors are present. It must be concluded that, from the data obtained here, there are not elements which permit to attribute a higher fitness to the individuals carrying pseudotumors.

It is to be noted that changes of fitness related to inbreeding have been found in some lines. But, these do not seem to also involve changes in pseudotumor incidence; therefore they are probably due to homozygosis of genes not linked to or recombining with *tu* genes.

Therefore, at this point of our study, the high incidence of the tumoral trait and its irregular unpredictable behavior cannot be explained by the orthodox mechanisms for fitness which are already known to operate in Mendelian populations.

This negative conclusion is consistent with the postulate of BARIGOZZI (1959, 1960) that there may exist a certain type of genic instability which plays a role in the determination of the tumor phenotype. The present work does not, however, indicate whether this instability in tumor incidence is due to changes involving one or several different genes or their location.

SUMMARY

Pseudotumor incidence was studied in 66 lines of *D. melanogaster* and in 189 of *D. simulans* maintained under different levels of inbreeding for eight or 14 generations. Pseudotumor incidence was fairly constant in most lines. In some lines incidence changed significantly, sometimes increasing, decreasing, or being irregular.

A study of some of the components of fitness such as fecundity, viability, sterility and rate of development did not demonstrate any effect of the presence of the pseudotumoral trait.

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RADIATION ANALYSIS OF A LECANOID GENETIC SYSTEM[1]

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IN 1921, SCHRADER first reported the unusual cytological system in a mealy bug *Pseudococcus nipae* (Mask.). Further work by SCHRADER (1923a) and HUGHES-SCHRADER (1935) resulted in an exact characterization of a system of chromosome behavior which has been named "lecanoid." These and other studies are discussed in HUGHES-SCHRADER's (1948) comprehensive review of coccid cytology. One set of chromosomes undergoes facultative heterochromatization in male embryos and becomes excluded from the genetic continuum at spermatogenesis.

Although a striking departure from the more conventional forms of chromosome behavior, the lecanoid system is not a passing accident but has sustained quite considerable evolution. It occurs throughout a series of diverse families including the primitive mealy bugs (Pseudococcidae), the cochineal dye insects, the lac insects, the soft scales (Lecaniidae), the asterolecanoids, and the conchaspids as well as the most specialized coccids, the armored scale insects (Diaspididae) in which it occurs as a dependent system (HUGHES-SCHRADER 1948; BROWN 1959).

In the lecanoid system both males and females are diploid. One haploid set becomes heterochromatic at the blastula stage in the male embryos but otherwise the chromosomes of the two sexes are not distinguishable. The heterochromatic set divides synchronously with the euchromatic but forms a conspicuous chromocenter in the resting nuclei. Chromosome behavior is usually the same in soma and germ line; however, according to recent observations (BROWN, unpublished; NUR, unpublished), heterochromatization is frequently absent from certain polyploid nuclei and may be restricted to the germ line in a few species.

At spermatogenesis (Figure 1), the first mitosis is equational for both types of chromosomes while the second is segregational, the heterochromatic set moving away from the euchromatic on a monopolar or highly asymmetric spindle. Only the euchromatic derivative forms sperm; the heterochromatic entity forms a highly pycnotic residue which is maintained during much of spermiogenesis. Chromosome comportment in the female is normal during ontogeny and

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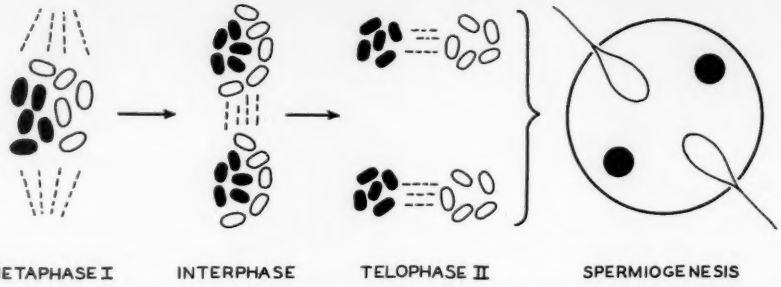

FIGURE 1.—Diagram of lecanoid spermatogenesis. The euchromatic and heterochromatic chromosomes divide equationally during the first division, and are segregated during the second. Spermatids contain all four products of the two divisions. Only the euchromatic derivatives form sperm; the densely pycnotic heterochromatic residues eventually disintegrate.

oogenesis. The presence of chiasmata in the bivalents may be taken to mean that recombination occurs by crossing over as well as by random assortment.

SCHRADER and HUGHES-SCHRADER (1931) believed the lecanoid system to be a stepping stone toward true haplodiploidy. The lecanoid male transmits only a single set of chromosomes and thus will breed as a haploid if this set were derived exclusively from either parent. SCHRADER and HUGHES-SCHRADER (1931) suggested that the heteropycnosis of one set of chromosomes indicates its genetic inertness; the male would therefore be a virtual haploid. HUGHES-SCHRADER (1948) further suggested that the heterochromatic set is of paternal origin. The present study was undertaken to test the validity of these two hypotheses by means of response to irradiation as suggested by P. W. WHITING's (1937, 1938) studies of the true haplodiploid system of Habrobracon.

MATERIALS AND METHODS

The laboratory stock culture of *Planococcus* (= *Pseudococcus*) *citri* (Risso) was obtained in August, 1957, from DR. D. C. LLOYD of the Commonwealth Institute of Biological Control, Fontana, California, and stock cultures were maintained by transferring eight to ten laying females each generation. The mealy bugs were grown on Irish potatoes, *Solanum tuberosum* L., wedged into glass jars with metal lids which were vented and screened with linen cloth. Since overcrowding may contribute to contamination, apparently by influencing the crawlers to explore rather than settle on the available potato, care was taken to prevent it. When any culture was terminated, it was autoclaved before opening. The laboratory was maintained at a mean temperature of 23°C.

Males and females of desired ages were obtained by withdrawing small numbers of females, usually four, at the start of oviposition and transferring them daily to fresh culture jars for a total duration of 9 to 11 days. Virgin females in the third instar were then later available from the daily-lay cultures before any of the males in the same jar had emerged from their cocoons as winged adults.

The males could either be collected for experimental crosses directly from their subculture jars or, since they do not feed after the end of the second instar, isolated in large numbers on cleansing tissue paper and maintained in corked glass vials. The transfers of individual insects were performed with slightly moistened camel hair brushes.

Except in the experiment designed to test the fertility of sons following paternal irradiation, all matings were made shortly after treatment and were initiated *en masse* on fresh potatoes to increase the likelihood of fertilization. Females were subsequently transferred to individual jars as soon as they began to lay their eggs. For irradiation males and females were placed in gelatine capsules. Controls were handled in the same way, including transportation to the radiation sites. X-irradiation was performed with a Phillips therapeutic X-ray unit and exposures were made with settings of 15 ma, 250 kvp, at a distance of about ten cm from the target and filtered with 0.5 mm aluminum. The Victoreen r-meter registered values of approximately 400r per minute. For the high-dosage series a cobalt^{60} chamber was used which delivered 3,333rep per minute at an opening of one inch. The radiation was performed at the Lawrence Radiation Laboratory through the courtesy of the Bio-organic Chemistry Group, University of California.

The progeny were counted as adults with the aid of a dissecting microscope. To avoid confusion, the individuals were either transferred to new potatoes for further crosses or destroyed during the counting process. Unhatched eggs were counted directly in the single ovisac left by the mother or if their number was large, the ovisac was transferred to a small sheet of filter paper and the embryos "floated out" on a larger area with a few drops of 95 percent ethyl alcohol.

About 3½ days following fertilization, gravid females contained a sufficient number of both male and female embryos for a cytological comparison to be made between them, i.e. those showing no signs of heterochromatization at late blastula and those at the same stage of development, but where heterochromatization of one set of chromosomes was just becoming apparent. Females of this age were stained and squashed in acetocarmine following fixation in BRADLEY's (1948) modification of Carnoy's fluid to which was added a very small amount of a saturated solution of ferric acetate in propionic acid. Embryos at the proper stage of development and with division figures could be selected without prejudice under low power and then analyzed for aberrations with an oil immersion objective; there were thus almost no unanalyzable embryos.

Life cycle of the mealy bug: Extensive embryonic development occurs prior to laying which commences about 4–6 days after mating and continues for nine to 11 days. The eggs hatch about nine days after laying and the freshly emerged crawlers wander around until they find a suitable place to feed. During the first and second instars, the males and females cannot be distinguished externally. Both are sedentary in habit and can be moved only with care not to break the very delicate feeding tube inserted into the plant. After the second instar the male stops feeding and undergoes metamorphosis inside a cocoon. Cracks and crevices are frequently chosen as sites for the pupation process. Spermatogenesis

is completed early in the third instar; spermatids develop synchronously and all sperm are mature when the male emerges as a winged adult 36–40 days after oviposition. He does not feed as adult, may mate with as many as 23 females (JAMES 1937) and dies within two to four days. He is extremely fragile, cannot be successfully anaesthetized, and must be handled individually and with care; he usually walks rather than flies, especially when freshly emerged.

The female can be mated soon after the last molt but will produce no offspring until full sexual maturity is attained. In her external morphology, she maintains the characteristics of the larval period and continues to feed until the end of oviposition. The sex ratio in JAMES' (1937) material was approximately 1:1; in our laboratory with a different strain and different cultural conditions, the ratio is approximately 0.67 males per female (NELSON-REES 1960). Fluctuations in the sex ratio and other reproductive characteristics of the female will be considered in the discussion.

RESULTS

Analysis of the genetic system of the mealy bug, *P. citri*, through its response to radiation consisted essentially of two parts, the influence on the progeny following treatment of either parent, and the transmission of induced effects. Both the genetic and cytological data will be presented before assessing the results in terms of the SCHRADERS' hypotheses. The data are in general quite consistent in their bearing on the hypotheses in question; however, the female mealy bug presents many complex problems in regard to variable sex ratio and variable and incomplete sexual dichronism of offspring. Though of secondary interest in regard to present purposes, the discrepancies stemming from such complexities require consideration. During the course of the present studies, a considerable number of radiation effects were discovered which merited detailed investigation, outside the scope of the present problem. Where it will help to clarify otherwise puzzling aspects of the present results, the progress to date in the subsequent studies will be summarized where pertinent in this section and the discussion.

X_1 response[1]

Maternal treatment: The average number of survivors per treated female is cited in Table 1 for two different experiments in which the age of the mothers differed. The survival curves, expressed in percentages of the control values of the same sex, are illustrated in Figure 2. The influence of maternal age on response to radiation has been the subject of extensive work (NELSON-REES 1960) which will be summarized in the discussion; the differences between the two experiments reported here are of the sort expected from the different maternal ages. In general, it may be concluded that maternal treatment results in marked lethality in both sexes of offspring with increasing dosage from 0 to 8,000r.

[1] In this report, the terms X_1 and X_2 will be used to indicate the first and second filial generations, respectively, in the experimental series.

TABLE 1

Average number of survivors per mother after maternal treatment

Dose	Series no. 1* n	Av. no. ♀♀	Av. no. ♂♂	5* n	Av. no. ♀♀	Av. no. ♂♂
0r	10	295.1	172.4	8	141.5	130.1
1,000r	21	45.7	59.9	11	123.4	134.0
2,000r	10	40.6	26.4	11	71.4	66.4
4,000r	7	4.1	1.4	10	19.2	12.1
6,000r	11	0.1	0.2	10	8.7	1.5
8,000r	10	0.2	0	10	1.8	0.1

* Females of age 33 days, series 1, and 42 days, series 5 when treated and mated.

Cytological examination following maternal treatments at 4,000 and 8,000r showed a marked increase in chromosomal aberrations in the embryos (Table 2, Figure 3). The normal diploid chromosome number for both sexes of *P. citri* is ten; in males, five chromosomes normally become and remain heterochromatic. There are no marked differences in chromosome size; the nucleolus organizer is near the middle of one chromosome which often appears disjunct at this region. Among the three control series, an unexpectedly high percentage of spontaneous alterations occurred in series B. The reason is unknown but was presumably not genetic since all series were drawn from the same stock culture. The types of aberrations are summarized in Table 3. Only those embryos were classed as

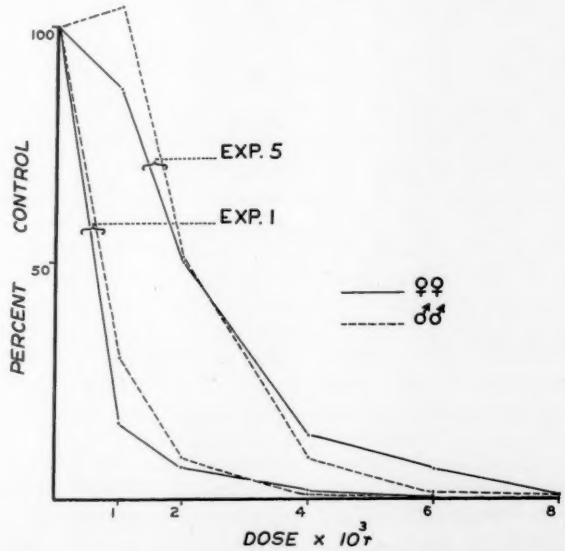

FIGURE 2.—Average percent survival to maturity of the X_1 following maternal treatment.

TABLE 2

Percentages of embryos with altered chromosome sets following maternal, paternal, and combined treatments

Treatment	Series	Age of mothers in days	No. of females	Male embryos				Female embryos		Young embryos	
				No. of embryos	Aberrations in H set	Aberrations in E set	Not distinguishable	No. of embryos	Aberrations present	No. of embryos	Aberrations present
Control											
	B	56–61	4	80	1.3	5.0	2.5	137	16.1	33	6.1
	C	45–46	9	100	0.0	4.0	0.0	26	3.8	30	0.0
	E	69	6	100	1.0	1.0	0.0	42	0.0	13	0.0
Maternal 4000r											
	B	56	1	37	0.0	32.4	10.8	29	37.9	22	50
	E	47	9	110	0.0	36.4	0.0	73	50.7	31	48.4
Maternal 8000r											
	C	43–46	19	114	1.7	37.7	1.8	42	71.4	15	93.3
	E	47–49	13	106	0.0	64.2	4.7	89	75.3	14	85.7
Paternal 8000r											
	D	55	3	52	90.4	0.0	0.0	28	92.9	11	90.9
	E	49	7	103	96.1	0.0	1.0	42	95.2	25	96.0
Combined maternal & paternal; each 8000r											
	E	47	4	37	24.3	0.0	..	4	100	3	100
				(both H & E: 73.0)							

mosaics in which one or more division figures were normal and one or more showed chromosome aberrations; this class presumably resulted from postzygotic disturbances. As would be expected, relatively more mosaics of altered and normal cells were found in the control than in the experimental series since treatment would usually result in damaged gametes rather than postzygotic changes. In the control series (Table 3), the difference between the sexes in the type of abnormality was a function of the sampling; relatively more fragmentation occurred in series B, in which a disproportionately large number of female embryos were scored, than in series C and E in which more males were examined. Furthermore in series B the fragmentation occurred preponderantly in the female embryos.

A comparison of maternal treatments of 4,000 and 8,000r (Table 2) shows that doubling the dose brought about a substantial increase in chromosomal changes in young and female embryos. In regard to the affected male embryos, those of series E increased with increasing dosage but at 8,000r those of series C were about equal to that expected following a dose of 4,000r. It may be suggested again that maternal age was the responsible factor; the mothers of series C were somewhat younger than those of series B or E.

The types of aberration found after female treatment (Table 3) consisted largely of elimination of single chromosomes, and fragmentation. Another class,

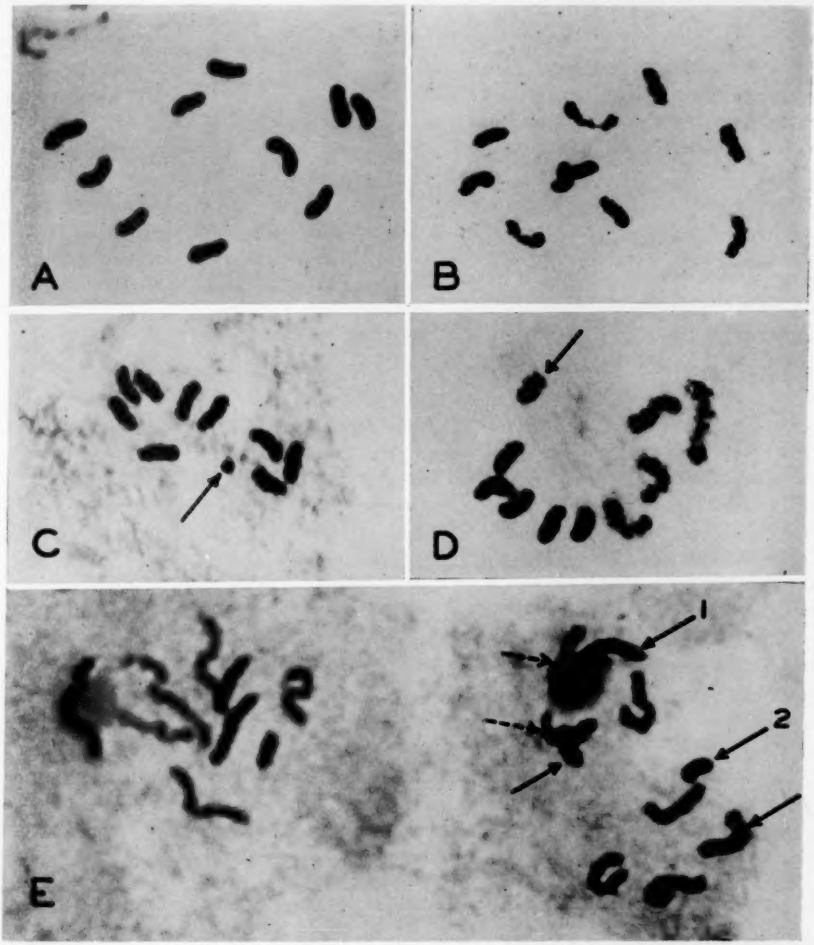

FIGURE 3.—Mealy bug chromosomes. A. Normal female set of ten chromosomes. B. Normal male set of ten chromosomes, five euchromatic, five heterochromatic, at late prophase. C. Nine chromosomes plus fragment in female embryo in which the euchromatic and heterochromatic sets cannot be differentiated. D. Male embryo; euchromatic set of four chromosomes and fragment (arrow). E. Male embryo, two adjacent cells; euchromatic set normal with typical subdivision of nucleolar chromosome (dashed arrows) on either side of submedian organizer; heterochromatic set (solid arrows) altered; of the four chromosomes, "1" is longer, "2" shorter than normal.

TABLE 3

Percentages of various chromosome aberrations in affected X_1 embryos cited in Table 2

		No. of embryos	9 or fewer chromosomes	9 or fewer chrom. & f.	10 chromosomes, size change*	10 chromosomes & f.	11 or more chromosomes
Control,	♂♂	11	54.5	9.1	0.0	9.1	9.1
non mosaic	♀♀ + young	26	15.4	11.5	7.7	7.7	7.7
Control, cont.,	♂♂	..	0.0	0.0	0.0	18.2	0.0
mosaic†	♀♀ + young	..	0.0	0.0	0.0	50.0	0.0
Maternal treat-	♂♂	163	43.7	13.5	12.9	17.2	3.7
ment, non mosaic‡	♀♀ + young	203	28.1	23.2	16.2	20.2	4.4
Maternal treatment,	♂♂	..	1.2	0.6	0.0	6.7	0.6
cont., mosaic†	♀♀ + young	..	1.0	0.0	0.5	6.4	0.0
Paternal	♂♂	97	7.9	40.3	25.2	20.1	6.5
treatment	♀♀ + young	139	0.0	32.7	22.5	42.8	2.0

* Decrease or increase estimated at 1/2 to 1/3 chromosome length; smaller changes not identifiable; chromosomes less than 1/2 classed as fragments.
† Only those embryos in which some division figures showed a normal complement.
‡ Doses 4,000r and 8,000r combined.

in which there were nine or fewer chromosomes plus fragments, included a range from those which had undergone elimination and fragmentation to those in which the fragmentation itself accounted for the missing chromosome(s). Other abnormal complements were identified by the decrease in length of a third to a half of one of the chromosomes. Some mosaicism was found in which part of the embryo was normal and the aberrations in the abnormal sector may have been of spontaneous origin. In several cases, embryos were mosaics in which all sectors showed abnormal complements; these were classified according to the most nearly complete complement in the embryo; for example, an embryo with two sectors, one of nine chromosomes plus fragments and the other of nine chromosomes alone, would have been classified according to the former complement.

The observed abnormalities did not account for the total percentage of dominant lethals. After both doses in question, survival was less than ten percent. The embryos which appeared to have normal complements and to be developing normally at the stages examined must therefore have been carrying cytologically undetectable dominant lethals. Deficiencies up to a fourth of the length of a chromosome would not have been recognizable.

Of special interest was the almost complete absence of bridges during early embryogenesis. In the entire series examined, only three bridges were found; when they did occur, they were sufficiently striking to be readily detected. The class of young embryos included immediately postzygotic individuals, and these did not show abnormal arrangements of nuclei that might have been expected from chromosome bridging or other mechanical effects. It may therefore be concluded that postzygotic breakage-fusion-bridge cycles arising from radiation-induced breaks contributed little or nothing to dominant lethality following maternal treatment. Bridging at oogenesis was much more common and was probably the source of a high proportion of embryos lacking one or more chromo-protein takes place. Thus it has been assumed that thymine starvation causes an irreversible

somes; the types of structural alterations responsible for oogenic bridging are not known.

In spite of the complexities and inconsistencies in dosage response, the cytological data clearly demonstrated that only the euchromatic set of the male embryos was damaged following maternal radiation. The number of embryos with aberrations in the heterochromatic set was small and did not differ significantly from the control (Table 2). In some cases it was not possible to determine in which set the aberration had occurred. The two sets could be differentiated and analyzed for aberrations only during the middle to late prophase stages. If only prometaphase and later stages were available the sets could not be differentiated and such cases were recorded as questionable. The nature of small fragments could be determined only at midprophase before contraction reduced them to unanalyzable dots. By far the greater majority of fragments were, however, clearly and unmistakably identifiable as either heterochromatic or euchromatic.

Paternal treatment: Male treatments were made with both X-rays, over a dose range from 0 to 16,000r, and radiocobalt from 0 to 150,000rep (Table 4, Figure 4). The highest dose at which survivors were obtained was 120,000rep; after treatment at 150,000rep or higher the males either failed to mate or, if they did, to ejaculate since no sperm were found in the females with which they had been placed.

The survival curves differed strikingly from those obtained from maternal treatment. That of the daughters consisted of three parts, the low-dosage range (0–16,000r) in which the number declined with increasing dosage, the intermediate-dosage range (16,000r–30,000rep) in which fewer than ten percent survivors occurred, and the high-dosage range (60,000–120,000rep) in which the number was maintained at about 40 percent of the control value.

The curve for the sons consisted of two parts. In the first, equivalent to the low and intermediate sections for the daughters (0–30,000rep), the number of males recovered was usually greater than the control value. In the second, the high-

TABLE 4

Average number of survivors per mother after paternal treatment

X-ray series no.	n	4 ♀♀	♂♂	n	17 ♀♀	♂♂	n	18 ♀♀	♂♂
0r	5	212.8	163.8	11	245.2	167.2	9	248.6	182.2
2000r	2	114.0	129.0	10	248.0	199.3	11	164.4	164.1
4000r	1	58.0	203.0	10	126.2	190.0	10	180.3	191.7
8000r	3	41.0	188.0	12	47.7	198.4	10	101.2	239.8
16000r	3	3.0	275.0	12	7.1	145.7	10	11.9	273.5
Radiocobalt series no.		I			III			VI	
0rep	10	225.7	75.8	10	279.1	180.6	11	354.1	201.6
15000rep	8	14.2	95.0	11	7.9	181.3	11	70.2	281.6
30000rep	10	24.2	130.9	10	6.2	126.3	11	6.1	254.9
60000rep	10	144.8	3.8	11	89.5	2.9	11	125.2	35.4
90000rep	..	.....	.....	9	84.8	0.2	11	126.4	0.7
120000rep	9	118.0	1.3	3	114.0	0	6	112.7	0

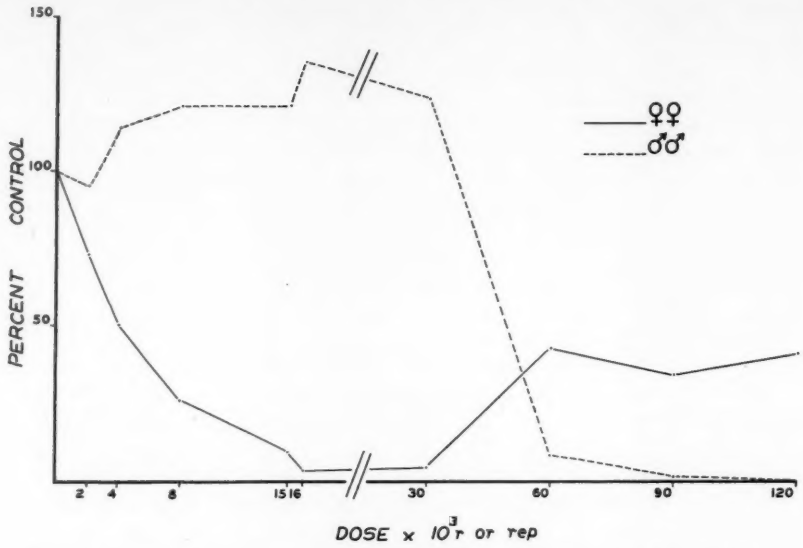

FIGURE 4.—Average percent survival to maturity of the X_1 following paternal treatment.

dosage range, the number of males was reduced abruptly to less than ten percent at 60,000rep and very few were obtained after treatments at 90,000 and 120,000rep.

Cytological examination of X_1 embryos after male treatment at 8,000r showed that over 90 percent had affected chromosome sets (Table 2, Figure 3). At the same dose, treatment of the fathers thus produced considerably more damage than treatments of the mother for all but the young embryos; however because of the complexities of assessing maternal treatment, this comparison is undoubtedly not completely fair. The paternal treatments yielded much more uniform results between the two experimental series and among the three classes of embryos than did the maternal.

The type of aberrations most commonly occurring after paternal treatment also differed. In relatively few embryos was a chromosome simply absent, and loss of a whole chromosome was usually accompanied by fragmentation (Table 3). Proportionately over twice as many female and young embryos as male carried ten approximately normal chromosomes plus one or more fragments. A large part of this discrepancy is no doubt attributable to the fact that the chromosomes studied in the two classes of embryos differed. In the male embryos, the aberrations occurred almost exclusively in the relatively more condensed heterochromatic set while in the female and young embryos all chromosomes are euchromatic; the residual section remaining after fragmentation would be more apt to be classified as a "fragment" rather than a "chromosome" if it occurred in a heterochromatic than a euchromatic set.

After paternal treatment of 8,000r, about 25 percent of the daughters survived yet only about five percent (average for series D and E) did not show some type of chromosomal change. All the observed changes were therefore not accompanied by dominant lethal effects.

The failure of appearance of mosaics of normal and aberrant complements after paternal treatment was probably a statistical effect. Irradiation resulted in about 95 percent aberrations, for the combined series D and E, for each of the three classes of embryos. A generous estimate of three percent of spontaneous aberration yielding mosaicism leads to an expected frequency of less than one case among the five percent which did not already show aberration.

Cytological studies of X_1 embryos and survivors are of special interest and are being continued as separate problems by CHANDRA and by NELSON-REES. Observations pertinent to the present study will be briefly summarized at this juncture. The few males surviving after high-dosage treatment clearly demonstrated the results of chromosome breakage in the heterochromatic sets; in most of the examples in which spermatogenesis was studied, the heterochromatic set consisted of a single very long chromosome, one or two normal chromosomes and several fragments (NELSON-REES unpublished). After high dosage treatment, one chromosome set appeared much like confetti in division figures in the X_1 embryos (CHANDRA unpublished). At anaphase the tiny fragments showed chromatin bridging which was difficult to analyze further but resulted in abnormal division figures and restitution nuclei. Of considerable interest was the observation that serious imbalance of the euchromatic and heterochromatic sets in the male embryos led to endomitosis and the formation of grossly polyploid embryos prior to death. In both the low and the high dosage series, pycnosis and other symptoms of necrosis were not apparent prior to gastrulation. CHANDRA (unpublished) further studied the cytology of the numerous females appearing after high-dosage treatment. Evidence of chromosome breakage was almost completely lacking. The females were usually triploid, but were occasionally diploid or diploid-triploid mosaics. The triploid females probably arose when the united polar bodies, which normally enter into the formation of the mycetocytes (SCHRADER 1923b), took over embryogenesis. The diploid females and diploid sectors of mosaics arose either from the second polar body or from zygotic derivatives in which the bridging of the damaged set had prevented its separation in mitosis and permitted the undamaged set to form independent daughter nuclei.

Cytological studies have not yet been made for the dose range of 15,000r to 60,000rep. Whether or not the formation of bridges is of gradual or abrupt onset, and the amount of damage to the zygotic derivatives required to permit triploid embryogenesis are at present open questions.

In conclusion, the results of paternal treatment demonstrate that there was little or no dominant lethality induced in male embryos in the dose range in which there were no mechanical disturbances in embryogenesis while the number of daughters decreased markedly over the same range. At the higher dose range, dominant lethality was induced at a high rate in the males while abundant chromatin bridging appeared in the embryos. As judged by the absence of aberra-

tions, the chromosomes of the females appearing after high-dosages were derived exclusively from their mothers. The damage to the male embryos stemming from paternal treatments was found almost exclusively in the heterochromatic set; similar results were obtained by CHANDRA (unpublished) after high-dosage treatments. The results of paternal treatment differed from those of maternal treatment by being in general much more consistent. In addition, after maternal treatment the percentage of dominant lethals was considerably higher than the percentage of embryos with detectable chromosome changes while the reverse was true following paternal treatment. Part of this difference is undoubtedly attributable to the completion of meiosis after radiation of the female.

X_2 and backcross progenies

Paternal treatment: The X_1 offspring obtained after various doses from 0 to 16,000r were allowed to cross *inter se* in the culture jars and the females were transferred before oviposition to individual cultures. X_1 males and females were also crossed individually to mates drawn from the stock cultures. The results of the three different sorts of crosses are given in Table 5 and the survival curves are pictured in Figure 5 as unweighted averages for the two experimental series. The differences between the two experimental series were probably attributable to cultural differences, such as the quality of potatoes, between the two tests as well as to the handling of the parental generation.

The X_1 *inter se* and the backcross of X_1 females to stock culture males gave essentially similar results. In both cases there was a steady decrease in the number of offspring of both sexes with increasing dose administered to the P_1 males. The percentage of survivors as calculated for each sex independently from its own control value was consistently lower for the males in both the X_1 *inter se* and the backcrossed X_1 females.

Cytological studies of the X_1 females surviving to maturity have been made

TABLE 5

Offspring of the X_1 obtained from paternal treatment

		X_1 *inter se*			X_1 ♀♀ × stock ♂♂			Stock ♀♀ × X_1 ♂♂		
			Av. no.			Av. no.			Av. no.	
Dose	Exp. no.*	n	♀♀	♂♂	n	♀♀	♂♂	n	♀♀	♂♂
0r	6	9	361.2	283.3	..	....	....	..	....	....
	18	8	254.2	168.9	..	....	....	..	....	....
2,000r	6	10	172.9	80.3	6	187.5	78.7	5	346.6	227.2
	18	9	189.6	69.8	9	204.3	153.3	4	276.5	192.0
4,000	6	11	86.4	16.9	5	60.8	13.0	8	251.5	166.5
	18	6	42.6	16.7	10	148.6	31.5	10	250.3	173.4
6,000r	6	15	58.9	8.6	8	60.4	14.5	7	266.3	151.3
8,000r	18	8	29.6	7.8	9	19.0	3.6	8	216.0	214.4
16,000r	6	13	6.2	2.6	7	17.4	2.0	5	358.0	207.2
	18	8	2.1	0.1	5	2.4	0.6	7	129.1	99.1

* In the P_1, males were slightly older when irradiated in exp. 18 than in exp. 6; treated males were mated to 29–34 day old females in exp. 6, to 44 day old in exp. 18.

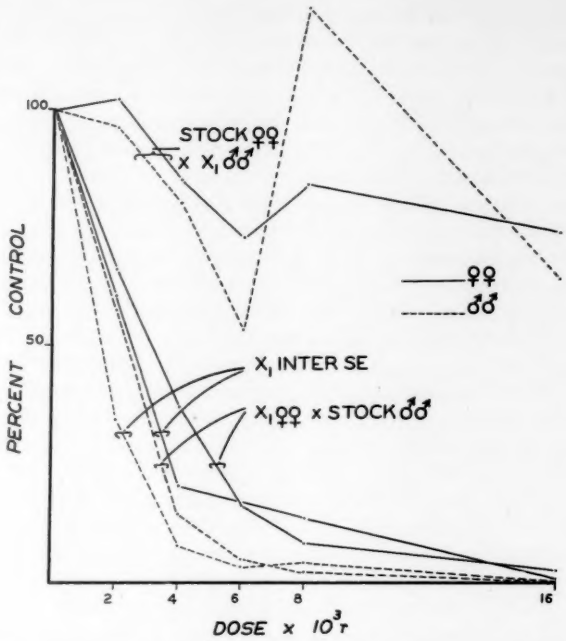

FIGURE 5.—Average percent survival to maturity of the X_2 and backcross progenies following original paternal treatment.

only to a limited extent; some but not all were seen to have altered chromosomes such as fragments, and others probably carried translocations which would not have been detectable unless grossly unequal. The partial sterility of the X_1 female has been assumed to be a consequence of the formation of duplication-deficiency gametes at oogenesis. Without prejudice as to the nature of the chromosomal changes involved, this type of dominant lethality may be referred to as meiotic-product lethality.

The X_1 males contributed nothing detectable to the results of the X_1 *inter se* since progenies similar to the X_2's were obtained when the X_1 females were backcrossed to the presumably normal stock culture males. However, when X_1 males were crossed with stock females, the total number of offspring decreased about 30 percent over the tested dose range (Figure 5). Statistical tests suggested by DRS. HENRY SCHEFFÉ and EVERETT R. DEMPSTER showed that the differences from control value were barely significant. The number of eggs which failed to hatch was not noticeably greater for this backcross than for the control series although a considerable increase in the number of unhatched eggs was invariably associated with both directly induced dominant lethality and that appearing in the X_2 or backcross progenies as meiotic-product dominant lethality. It seemed

likely, therefore, that if the X_1 males differed from the controls, they did so not in their transmission of lethals but in reduced vigor expressed as failure to fertilize effectively. These experimental series with the X_1 males gave the only results which seemed to contradict one of the hypotheses being tested. On the other hand, the difference from control may have stemmed simply from the very marked fluctuations in total productivity of the female mealy bug. The situation seemed, therefore, to merit further analysis.

A mealy bug female may mate either simultaneously (NELSON-REES 1959) or successively with more than one male. By giving the female more than one mate, compensation could be made for any lack of vigor shown by the individual males. An experiment was set up in which single females were given one, three, or six males for both the control series and the experimental. The results are summarized in Table 6. Females seemed to fail more often to produce any offspring when provided with only one male, but there were no noteworthy differences in this regard between the control and experimental series. Although there was here probably no cause and effect relationship, productivity declined in the control series with increase in the number of males but in the experimental series it varied apparently at random. The percentages for the experimental females were calculated from the productivity of the control female mated to the same number of males and for the two sexes independently. The fact that the percentages in the experimental series were usually over a hundred was probably attributable simply to the already noted decline in productivity of the control females with increase in number of males. In this experiment care had been taken to control more precisely the age of the mother as other work in our laboratory had meanwhile shown that a few days difference in the age of a sexually mature female could materially influence her total productivity (NELSON-REES 1960). As in the first experiment, there was no increase in the number of unhatched eggs in the experimental series. Within the limits of the second experiment, the males obtained after paternal treatment were as effective in mating

TABLE 6

Offspring of X_1 males (from paternal treatment) mated in different numbers to to individual stock females

Dose*	No. of males†	No. females Laying	No. females Not laying	Av. no. offspring‡ ♀♀	Av. no. offspring‡ ♂♂	Percent controls§ ♀♀	Percent controls§ ♂♂
0r	1	21	4	175.1	124.1	....	....
	3	23	0	167.6	106.4	....	....
	6	23	0	144.2	104.3	....	....
8,000r	1	21	1	163.7	138.3	93.5	111.5
	3	19	1	183.8	132.0	109.7	124.0
	6	16	1	172.9	137.5	119.9	131.8
16,000r	1	19	4	177.3	130.5	101.2	105.2
	3	24	1	140.5	112.8	83.9	106.0
	6	22	1	179.7	130.8	124.6	125.4

* To the P_1 male.
† Number of males placed with the individual stock females.
‡ Of those laying.
§ For the comparable 0r cell: sex and no. of males.

as those taken from stock cultures. The converse experiment, of mating individual males to varying numbers of females, was not performed.

The few X_1 males appearing after high dosage treatments (90,000rep) presented quite another picture. On mating, all have proved to be sterile; some were found on dissection to have defective testes but in others, the only detectable developmental abnormalities were some malformed spermatids (NELSON-REES unpublished). According to the cytological studies of CHANDRA (unpublished) described above, the high-dosage males must have survived an early embryogeny which was highly disturbed. The absence or failure of normal development of the testes may perhaps have been a reflection of greater derangement in the first cells of the germ line than elsewhre in the embryo. The converse condition, viable cells in the germ line but not in the soma, would not permit survival. A further possible cause of the observed sterility will be considered in the discussion.

Maternal treatment: Only a limited number of crosses have been made with X_1 individuals obtained following the more severe maternal treatments (Table 7). When X_1 females were tested, the number of offspring of both sexes declined with increase in the original dose and the males were considerably more affected than the females. The results were in general similar to those obtained with X_1 females following paternal treatment but considerably less severe at the same doses. Only three X_1 males were tested, two after 4,000r and one after 8,000r; on crossing to stock culture females, each gave an essentially normal progeny. Although the number of sons from male no. 1 was low, there was no corresponding increase in unhatched eggs as would be expected if lethal factors were the reason. Several daughters of each X_1 male were next tested and these all yielded normal progenies, again with no or few unhatched eggs.

DISCUSSION

Sex determination: The sex ratio of the total offspring of a female mealy bug

TABLE 7

Offspring of X_1 males and females obtained from maternal treatment

a. Average number of offspring of X_1 females

Dose	n*	Av. no. ♀♀	Av. no. ♂♂	Percent control ♀♀	Percent control ♂♂
0r	8	254.2	168.9	...	...
4000r	10	187.9	100.2	73.9	59.3
6000r	10	179.0	67.0	70.4	39.7
8000r	6	159.7	39.5	62.8	23.4

b. Offspring of three X_1 males and of their daughters

	X_1 male no.†	No. offspring		n‡	Av. no. ♀♀	Av. no. ♂♂
4000r	1	208	47	4	189.0	122.5
	2	243	212	5	269.6	167.0
8000r	3	372	123	7	268.7	193.6

* Mated with stock males.
† Mated with stock females.
‡ Daughters of X_1 males; mated with stock males.

may vary widely from mother to mother and is also markedly influenced by maternal age at mating and by environmental conditions (JAMES 1937, 1938). HUGHES-SCHRADER (1948) suggested, therefore, that sex determination is under weak genetic control, easily modified by other factors. No sex chromosomes have been identified in any of the lecanoid examples studied and, until the onset of heterochromatization in those embryos destined to be male, all normal embryos have identical chromosome complements.

NELSON-REES (1960) has confirmed JAMES' results and has also found, from a study of the daily-laying series, that the mealy bug exhibits an incomplete sexual dichronism which varies with the age of the mother. For example, females mated at 46 days of age produced a preponderance of male offspring during the first few days of oviposition while those mated at 56 days yielded mostly females during the same period. By choosing mothers of appropriate age, almost any type of brood pattern could be produced. This variation in sexual dichronism with age was independent of the influence of maternal age on the sex ratio of all her offspring since the increase in proportion of males did not begin until the mother was about 60 days old. Total productivity was also influenced by maternal age, rising to a peak some ten days after the last molt.

In NELSON-REES' radiation experiments, sex ratios among the offspring as well as total survival were markedly influenced by the age of the mother at the time of treatment and mating. These results were explained on the assumption that the oocytes destined to yield male or female embryos were differentially sensitive to radiation because they were, as indicated by the sexual dichronism, at different stages of maturation when treated. Similarly the varying effect of treatment on total productivity was a reflection of varying proportions of oocytes at different stages of development. The sort of changes in the sensitivity of oocytes presumed for the mealy bug have been demonstrated by ANNA R. WHITING (1945a,b) in Habrobracon and in Sciara by BOSEMAN and METZ (1949). Although the experiments of NELSON-REES have not provided an answer to the problem of sex determination in the mealy bug, they have revealed unsuspected intricacies in the process which must be taken into account in any experimental work.

The SCHRADERS' *hypotheses:* With the complexities described above in mind, we may now consider the bearing of the present results on the two hypotheses of the SCHRADERS. According to that which we shall refer to as hypothesis I, the heterochromatic set is of paternal origin. That the male mealy bug breeds as a haploid, transmitting only the euchromatic set, has been so well established by numerous cytological observations that it should be considered a fact; however, the bearing of certain experimental results on the conclusions from the cytology will be considered below. According to hypothesis II, the heterochromatic set is genetically inert and the male mealy bug a virtual haploid. With the exception of the requirement of fertilization in the mealy bug, the genetic system expected is thus exactly that of a typical haplodiploid insect in which the haploid males arise from unfertilized eggs. This statement should not be taken to imply that

secondary, residual, or special genetic or physiological activity is or is not carried on by the heterochromatic set.

Genetic factors in a haplodiploid organism are expected in general to be sex linked in the same fashion as X chromosome markers in an XX-XY mechanism. The simplest genetic test for the existence of such a system is that of the reciprocal cross but we have had available in the mealy bug neither genetic nor chromosomal markers.

The cytological studies after maternal and paternal treatments demonstrated quite convincingly that the heterochromatic set is of paternal origin and hypothesis I of the SCHRADERS is thus confirmed (Table 8a,c). The results after maternal treatment also indicated that radiation did not itself detectably induce heterochromatization because the effects of treatment were limited to the euchromatic set.

The demonstration of the genetic inertness of the heterochromatic set (hypothesis II) depended primarily on the failure of paternal treatment to induce dominant lethality in male offspring at doses severe enough to kill off more than 90 percent of the daughters (Figure 6A,B). Over 90 percent of the embryos of both sexes had altered chromosomes after paternal treatment at 8,000r. The chromosomes of embryos of both sexes sustained approximately the same amount of damage but in the male embryo the damage was restricted to the heterochromatic set. It may therefore be concluded that dominant lethality cannot be induced by uncomplicated damage to the heterochromatic set (Table 8b). The lethality at higher doses may be explained by the induced chromatin bridging and the consequent disturbances in embryogenesis. If bridging which resulted

TABLE 8

Summary of bearing of results on the SCHRADERS' *hypotheses*

	Hypothesis I (Heterochromatic set paternal)	Hypothesis II (Heterochromatic set inert)
Paternal treatment—X_1		
a. Induced aberrations in heterochromatic set in male embryos	confirm	....
b. No dominant lethality in sons at lower doses (0r–30,000rep)	....	confirm
Maternal treatment—X_1		
c. Induced aberrations in euchromatic set in male embryos	confirm	....
d. Lethality induced in two sexes varying with maternal age (recessive lethal component not identifiable)	....	neutral
Paternal treatment—backcross		
e. X_1 males about as vigorous and fertile as stock males	....	1st exp. contradict 2nd exp. confirm
Paternal or maternal treatment—X_2 and backcross		
f. X_1 daughters yield proportionately fewer sons (recessive lethal component)	....	conform

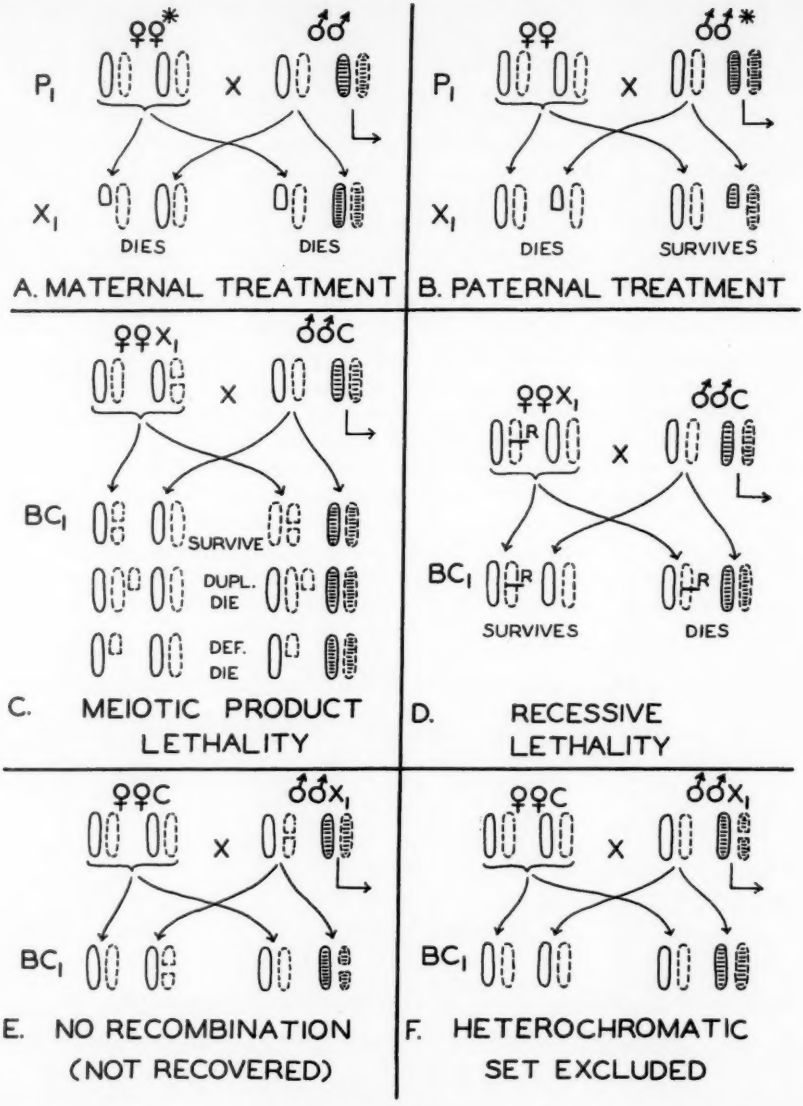

FIGURE 6.—The results expected from various crosses according to the hypotheses of SCHRADER and HUGHES-SCHRADER that the heterochromatic set (shaded chromosomes) is of paternal origin and is genetically inert. (See FIGURE 1 for diagram of lecanoid spermatogenic sequence). (C, stock culture).

in lethality were a regular sequel to chromosome breakage, dominant lethality in the X_1 males would have been expected to increase immediately and proportionately with paternal dosage regardless of the inertness of the heterochromatic set.

Further support for the validity of hypothesis II comes from the data on the progenies of X_1 females obtained from either paternal or maternal treatment. In these the proportion of males was consistently reduced to a greater extent than that of the females. In addition to the meiotic-product dominant lethals which kill both males and females (Figure 6C), the males would be expected to be killed also by recessive lethals if the heterochromatic set were inert (Figure 6D). Differential sensitivity of the oocytes was presumed to be responsible for much of the fluctuation in sex ratio among the progenies following upon maternal treatment (Table 8d); this factor would not be important in the reproduction of the X_1 females which were untreated. However, two other possibilities must be taken into consideration. The damage to the chromosomes carried by the X_1 females may have grossly influenced sex determination in favor of daughters in a manner analogous to the modifications in sex ratio induced by maternal aging and environmental factors. Secondly, the meiotic process itself may vary with the age of the mother; less regular pairing and assortment would thus have yielded a larger proportion of unbalanced gametes in those oocytes in the X_1 females destined to yield sons. Although both of these latter alternatives seem unlikely, neither can be ruled out at present; the data from the X_2 progenies, and those from backcrosses to stock culture males of X_1 females, should be regarded as conforming to the expectations of hypothesis II rather than directly confirming it (Table 8f).

The data from the first experimental crosses with X_1 males derived from paternal treatment indicated that these may have been hindered in effective fertilization. These results implied that damage to the heterochromatic set had a deleterious effect on the males and thus contradicted hypothesis II. Within the limits of a second, more extensive test, no significant difference between the

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A. Dominant lethals induced by maternal treatment(*) would be equally effective against sons and daughters.

B. Dominant lethals induced by paternal treatment(*) would kill only daughters; in the sons, the damaged chromosomes would be heterochromatic, inert and, without secondary complications, lead to no lethality.

C. A broken chromosome, otherwise not lethal, would be expected to result in some duplication-deficient gametes at oogenesis and lead to dominant lethality in both sexes. (See also E).

D. A recessive lethal (R) would be expected to kill the 50 percent of sons receiving it because the corresponding locus in the heterochromatic set is inert.

E. Parts of a broken chromosome will not be subject to recombination during spermatogenesis; thus no meiotic-product lethality will be expected. Altered chromosomes transmissible through the male have not yet been observed directly nor recovered, presumably because of associated recessive lethals. (See also F).

F. Damaged paternal derivatives will not be transmitted by sons; normal segregation at spermatogenesis of badly damaged heterochromatic sets (90,000rep) observed by NELSON-REES (unpublished).

control and experimental males was detected. The second experiment thus offers further confirmation of hypothesis II (Table 8e). In his original work with the mealy bug, SCHRADER (1921) believed that the Y chromosome(s) of the male was represented by the heterochromatic set; according to subsequent work (see HUGHES-SCHRADER 1948) the heterochromatic set does not form sperm and is therefore not transmitted similarly to a Y chromosome. SCHRADER's first interpretation should be kept in mind however in regard to possible genetic activity. The heterochromatic set or certain regions in it might thus have a function similar to the Y chromosome of *Drosophila melanogaster* which is necessary for the fertility but not the viability of the male (STERN 1929). Our results do not completely rule out the existence of such factors; they do indicate that there can be no more than a few active regions or the segmental losses revealed by the cytology of the low dosage X_1 male embryos (paternal treatment) would frequently lead to sterility. Part of the sterility of X_1 males following high-dosage paternal treatment may possibly be attributable to damage to fertility factors (NELSON-REES unpublished), as well as to the developmental derangements already suggested.

Confirmation of the numerous cytological studies of lecanoid spermatogenesis comes from the failure of X_1 males to yield meiotic-product lethals after either maternal or paternal treatment in which regard they differed strikingly from their sisters. The first spermatogenic division is equational for all chromosomes while the second segregates the euchromatic from the heterochromatic set; there would thus be no opportunity for recombination to produce the duplication-deficiency gametes presumed responsible for the meiotic-product lethality. The expectations from cytology would here remain the same whether the heterochromatic set were genetically inert or not and whether of paternal or maternal origin (but not if of mixed origin).

In studies of spermatogenesis in X_1 males following high-dosage treatments, NELSON-REES (unpublished) has observed the segregation of the damaged heterochromatic set, including fragments, from the euchromatic. Although insufficient yet to prove that all fragments, particularly the very small, will always behave regularly, these observations do confirm the expectations from the normal cytology that no recombination occurs between the two sets (Figure 6F).

Since the heterochromatic set is genetically inert, it is perhaps not surprising that we have not yet succeeded in finding cytologically demonstrable rearrangements transmissible through the male (Figure 6E). The three X_1 males from higher-dosage maternal treatment (Table 7) failed to transmit to their daughters the basis for meiotic-product lethality. Other, more extensive attempts also failed when X_1 females, following paternal treatment, were selected on the basis of reduced progenies. It seems likely, therefore, that many of the chromosomal changes were accompanied by recessive lethals and recovery of those viable in the male will require further trials with more stringent screening methods.

The hyperabundance of X_1 males: The increase in number of the X_1 males which appeared after paternal treatments from 4,000r to 30,000rep has been consistent enough to be regarded as more than a reflection of the vagaries in total productivity of the female mealy bug. The mealy bug embryo is at a fairly late

stage of development, with legs, antennae, and mouth parts quite well formed, at the time the egg is laid. Following treatment, embryos may die at any stage from gastrula onwards. On the assumption that the embryo continues to draw nutrients from its mother during most of its ovarian development, its death prior to oviposition would allow the mother to divert materials to the production of additional eggs and embryos; thus the total number of initial zygotes would be increased over that of the controls. If no lethality were induced in the sons, their total number should be increased over that of the controls while a higher percentage of females would survive than would otherwise occur.

Effectiveness of paternal and maternal treatments: Although it was outside the scope of the present problem to determine the relative sensitivity to irradiation of oocytes and sperm, a few of the striking contrasts between the two types of treatment will be briefly noted. At similar doses up to 8,000r, maternal treatments resulted in a much more drastic decrease in number of daughters. The effects on the sons cannot, of course, be compared. Cytological examination indicated considerably more apparent derangement in the chromosomes of X_1 embryos after paternal than after maternal treatment. Thus the surviving daughters would presumably have carried fewer chromosomal rearrangements after maternal than after paternal treatment. This difference may have been reflected in the tests of the X_1 daughters which yielded very much more reduced progenies after original paternal than after original maternal treatments at comparable doses. At any rate, the ratio of directly induced to delayed dominant lethality was obviously much higher after maternal than paternal treatment. It seems likely that part of this difference may be attributed to the screening effect of oogenesis which was completed after irradiation. Frequent elimination of smaller fragments during oogenesis would yield apparently normal chromosome complements which in reality were sufficiently deficient to be immediately lethal. The surviving females would therefore have fewer altered complements capable of yielding meiotic-product lethals.

Delayed dominant lethality and the holokinetic chromosome: In Drosophila, translocations will lead to the formation of duplication-deficient gametes; these in turn will result in the formation of inviable zygotes unless combined with a gamete carrying the complementary duplication and deficiency (MULLER and SETTLES 1927). Thus an X-ray induced translocation, though in no way lethal in itself, will yield dominant lethality in the X_2 generation. This type of delayed dominant lethality, which we have referred to as meiotic-product lethality, should be enhanced proportionately over that directly induced in organisms with holokinetic chromosomes. In a typical chromosome, a break in a chromosome arm will lead directly to a deficiency because the acentric portion will not be maintained, and the deficiency will often be sufficiently great to cause immediate dominant lethality. In a holokinetic chromosome, a similar break will lead to the formation of two fragments both of which may be maintained; there will thus be no deficiency and no immediate dominant lethality. Pairing behavior and assortment of the fragments at subsequent meioses is expected to be irregular, and at that time to lead to the formation of duplication-deficiency gametes.

With either type of chromosome, the initiation of a breakage-fusion-bridge cycle (McCLINTOCK 1938) might be expected to aggravate genetic inbalance. Bridges during cleavage have been observed after parental treatment in Habrobracon (ANNA R. WHITING 1945a,b) and in Drosophila (SONNENBLICK 1940). Of particular interest in the present case are the conclusions of CATCHESIDE and LEA (1945) in regard to the loss of the "largely inert" Y-chromosome of *Drosophila melanogaster* from the embryo following paternal irradiation; since the loss of the Y nearly always results in lethality, "The explanation presumably lies in the mechanical difficulties experienced by a dividing cell in which a dicentric bridge occurs as a result of sister union between chromatids at the breakage point." Such bridging, if it had occurred in the genetically inert heterochromatic set of male embryos of the mealy bug after low and moderate doses of paternal irradiation would probably have led to considerable lethality and obscured the demonstration of genetic inertness.

Under certain circumstances, coccid chromosomes are capable of forming bridges. In Steatococcus, bridges appear in spermatocytes but not in embryos after X-ray treatment (HUGHES-SCHRADER and RIS 1941). The bridges in the mealy bug embryos after high dosage paternal treatments have been difficult to analyze critically because the chromosomes have been severely fragmented; it is not known whether they were in any way the immediate result of the breakage; they may have been the product of chromosome stickiness induced by multiple fragmentation or formed by the irregular separation of small ring fragments (CHANDRA unpublished).

Evolutionary significance: The pseudococcids are a relatively unspecialized group at or near the start of an evolutionary series which extends to the armored scale insects (BALACHOWSKY 1942). Four chromosomal systems are now known for this series: the primitive XX-XO mechanism found so far only in the pseudococcid genus Puto (HUGHES-SCHRADER 1944, 1948); the lecanoid system (HUGHES-SCHRADER 1948; BROWN 1959); and, restricted to the armored scales, the diaspidid (BROWN and BENNETT 1957; BENNETT and BROWN 1958) and the Comstockiella systems (BROWN 1957). In the diaspidid system of the armored scales, the males become true haploids after the elimination of the paternal set at late cleavage. The paternal origin and primary inertness of the heterochromatic set of the mealy bugs thus make the lecanoid system appear to be a likely progenitor of true haplodiploid systems as originally suggested by SCHRADER and HUGHES-SCHRADER (1931), and more recently discussed by BROWN (1958). Evidence which is continuing to be accumulated indicates, however, that the Comstockiella system has been of major importance in the evolution of the armored scales (BROWN unpublished). In some species of armored scales, the Comstockiella and lecanoid systems may occur in different cysts of the same testis and are therefore indistinguishable until spermatogenesis; thus in certain respects the two systems would be expected to have the same evolutionary potentials.

SUMMARY

In the lecanoid chromosomal system, as exemplified by the mealy bug, *Planococcus citri* (Risso), one chromosome set becomes heterochromatic during embryogeny of the male and is maintained as such during development. At spermatogenesis, the first division is equational for both the euchromatic and heterochromatic chromosomes which are segregated from each other in the second; only the euchromatic derivatives form sperm. SCHRADER and HUGHES-SCHRADER suggested that the heterochromatic set is genetically inert and HUGHES-SCHRADER suggested that it is of paternal origin. Radiation studies were undertaken to test the SCHRADERS' hypotheses.

After paternal irradiation, the induced aberrations appear in the heterochromatic set of the male embryos while they occur in the euchromatic set after maternal treatment. HUGHES-SCHRADER's hypothesis of the paternal origin of the heterochromatic set is therefore confirmed.

After paternal irradiation of doses up to 30,000rep, dominant lethality is induced in daughters but not in sons. There is thus a clear picture of the failure of damage to the heterochromatic set to produce a detectable genetic effect and the hypothesis of the SCHRADERS in regard to its inertness is confirmed. These results do not rule out a secondary genetic or physiological function of the heterochromatic set. No breakage-fusion-bridge cycles, which would complicate the results whether the heterochromatic set were inert or not, were observed in the cytological studies after low-dosage treatments.

Further support for the hypothesis of inertness is provided by tests of X_1 males derived from fathers treated at 8,000 and 16,000r; these are as effective in fertilizing as their controls.

Because of the diffuse nature of the spindle attachment of coccid chromosomes, fragments can perpetuate themselves. Thus a complete break need not lead to a deficient chromosome. However, at subsequent oogenesis, the breakage products and other rearrangements would be expected to contribute to the formation of duplication-deficiency gametes. These expectations are borne out by the reduction in progeny, proportionate to original dosage, of X_1 females after either maternal or paternal treatment. In these progenies, the reduction in male offspring is proportionately greater than in the female; if the heterochromatic set is inert, the males would be expected to succumb to recessive as well as dominant lethals.

After maternal treatment, both sexes diminish with increasing dosage but the results are complicated by the effect of maternal age on the sequence in which the two sexes of offspring occur during oviposition (sexual dichronism) as well as on total progeny.

At high-dosage paternal treatments, 60, 90, and 120,000rep, the number of sons is drastically reduced while the number of daughters is about 40 percent of the controls. Cytological studies by CHANDRA (unpublished) indicate that the chromosomes are grossly damaged; chromosome bridging is prevalent and results in abnormal embryogenesis; the female survivors, triploid, diploid, and mosaic,

have undamaged chromosome sets which are therefore completely of maternal derivation.

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DIRECT AND INDIRECT TRANSFER OF THE "SEX-RATIO" CONDITION IN DIFFERENT SPECIES OF DROSOPHILA

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SEVERAL geneticists have been trying to elucidate the causes of the maternally transmitted abnormal sex ratios in Drosophila. It was found, in different species of Drosophila, that certain females collected in nature give exclusively or almost exclusively female progenies. This was found to depend on cytoplasmic inheritance (MAGNI 1952; CAVALCANTI and FALCÃO 1954; CARSON 1956; MORIVAKI and KITAGAWA 1957; MALOGOLOWKIN 1958). Furthermore, in the case of *Drosophila prosaltans*, *D. willistoni* and *D. paulistorum*, there are indications that the cytoplasmic factors are under partial control of chromosomal genes (CAVALCANTI and FALCÃO 1954; MALOGOLOWKIN 1958), which influence the resistance of the "sex-ratio" females to this condition. The hatchability of the eggs in "sex-ratio" females, is less than 50 percent as shown by MAGNI (1953), MALOGOLOWKIN (1958) and KAPLAN and MACEDO (1960). Unhatchable eggs can easily be distinguished 2–4 hours after being laid (MALOGOLOWKIN and POULSON 1957).

Transfer of the "sex-ratio" condition was obtained when the oöplasm for abnormal eggs was injected into the abdomen of virgin females from normal strains. This showed that the abnormal sex ratios, at least in *Drosophila willistoni* and in *D. equinoxialis*, are caused by infective agents which can be transmitted from generation to generation (MALOGOLOWKIN and POULSON 1957; MALOGOLOWKIN, POULSON and WRIGHT 1958; MALOGOLOWKIN 1959). The "sex-ratio" condition was also transferred from *D. willistoni* into *D. equinoxialis* (MALOGOLOWKIN 1960a; MALOGOLOWKIN, CARVALHO and DA PAZ 1960) and into *D. melanogaster* (SAKAGUCHI and POULSON 1960). The elimination of the "sex-ratio" condition by temperature treatments was obtained by MAGNI (1953) in *D. bifasciata* and by MALOGOLOWKIN (1959) in *D. equinoxialis*.

All these evidences suggest the hypothesis that the "sex-ratio" condition was caused by an infective particle, transmitted through the egg cytoplasm. In 1960, POULSON and SAKAGUCHI made the brilliant discovery that the infective particle, in the case of *D. willistoni*, was a microorganism of the genus Treponema, which could be found in the hemolymph of "sex-ratio" females but not in normal strains. The same was demonstrated for *D. equinoxialis*, but in the case of *D. bifasciata* no Treponema was found in the hemolymph of the "sex-ratio" females (MALOGOLOWKIN 1960b).

The purpose of this article is to describe the results of new experiments on the interspecific transfer of the "sex-ratio" condition.

MATERIALS AND METHODS

All females were injected by L'HÉRITIER's method, as described by MALOGOLOWKIN, CARVALHO and DA PAZ (1960). The strains of *D. willistoni* and *D. equinoxialis* were the same as those used by these authors. The following strains were also used:

1. *D. paulistorum* from Belem, Para, Brazil, collected by PROF. TH. DOBZHANSKY in 1952.
2. *D. nebulosa* from Rio de Janeiro, Brazil.
3. *D. prosaltans* from Ceara, Brazil, collected by PROF. A. G. L. CAVALCANTI in 1958.
4. *D. bifasciata* (one normal and one "sex-ratio" strain) from Pavia, Italy, kindly sent by DR. G. MAGNI in 1959.

RESULTS

Table 1 shows the results of the attempts of transfer by injection. Only one interspecific transfer, from *D. willistoni* into *D. nebulosa*, succeeded, as shown in Tables 2 and 3 (HSR1). The incubation period was of 9–13 days, the same as

TABLE 1

Females injected in the experiments of transfer of the "sex-ratio" condition between species of Drosophila

"SR" host	Recipient	Number of injected females	Results*
D. willistoni	*D. paulistorum*	30	—
D. willistoni	*D. bifasciata*	19	—
D. willistoni	*D. nebulosa*	13	+
D. willistoni	*D. willistoni*	17	+
D. equinoxialis	*D. paulistorum*	17	—
D. equinoxialis	*D. nebulosa*	16	—
D. equinoxialis	*D. prosaltans*	46	—
D. bifasciata	*D. willistoni*	67	—
D. bifasciata	*D. nebulosa*	6	—

* + Indicates that infection was obtained. — Indicates that infection did not occur.

TABLE 2

Sex ratios of the progeny of 13 D. nebulosa *females injected with the supernatant from* D. willistoni *"sex-ratio" females (HSR1), and of 16 females of* D. nebulosa *injected with the supernatant from* D. equinoxialis *"sex-ratio" females (HSR2)*

HSR1			HSR2		
Days of oviposition after injection	Number of first generation of flies	Percent males	Days of oviposition after injection	Number of first generation of flies	Percent males
1–9	500	45.0	1–9	497	45.3
9–13	1097	45.8	9–13	147	53.7
13–17	2263	14.2	17–19	137	35.8
19–21	780	16.3	19–21	342	46.2
21–39	1622	1.0	21–61	1409	46.4

when *D. willistoni* was used as the recipient (MALOGOLOWKIN, POULSON and WRIGHT 1958; MALOGOLOWKIN, CARVALHO and DA PAZ 1960).

Females of *D. nebulosa* injected with the supernatant from a macerate of "sex-ratio" females of *D. equinoxialis* were not infected, as can be seen in Table 2 (HSR2), but a transfer succeeded after the 21st day of injection, as can be seen in Tables 4 and 5 (HSR3). For this, the artificially produced "sex-ratio" strain

TABLE 3

Number of unisexual progenies from daughters of D. nebulosa *females injected with the supernatant from* D. willistoni *"sex-ratio" females (HSR1)*

Days of oviposition after injection	Daughters tested	Unisexual progenies
1–9	11	. .
9–13	39	2
13–17	41	37
17–19	. .	. .
19–21	31	19
21–37	71	70

TABLE 4

Sex-ratio in the progeny of 21 D. nebulosa *females injected with the supernatant from* D. willistoni *females of a strain made "sex-ratio" by a transfer from* D. equinoxialis *"sex-ratio" (HSR3), and of 19 females of* D. willistoni *females injected with the same supernatants (HSR4)*

HSR3			HSR4		
Days of oviposition after injection	Number of first generation of flies	Percent males	Days of oviposition after injection	Number of first generation of flies	Percent males
1–9	227	46.3	1–9	201	35.3
9–13	413	50.4	9–13	156	35.2
13–17	281	44.5	13–17	181	17.1
17–19	79	37.9	17–19	82	8.5
19–21	146	37.7	19–21	144	32.6
21–47	209	25.8	21–47	188	10.1

TABLE 5

Number of unisexual progenies from daughters of D. nebulosa *females injected with the supernatant from* D. willistoni *females of a strain made "sex-ratio" by a transfer from* D. equinoxialis *"sex-ratio" (HSR3), and unisexual progenies from* D. willistoni *females injected with the same supernatant (HSR4)*

Days of oviposition after injection	HSR3 Daughters tested	Unisexual progenies	Days of oviposition after injection	HSR4 Daughters tested	Unisexual progenies
1–9	12	—	1–9	26	—
9–13	3	—	9–13	19	5
13–17	53	—	13–17	55	33
17–19	6	—	17–19	49	42
19–21	53	—	19–21	53	44
21–47	62	5	21–47	107	107

of *D. willistoni*, originated by transfer from *D. equinoxialis*, as previously reported (MALOGOLOWKIN, CARVALHO and DA PAZ 1960), was used as a source of the "sex-ratio" factor. Supernatant from a macerate of females from the 17th generation of such "sex-ratio" strains was injected into 21 virgin females of *D. nebulosa* from a normal strain. Only five of them gave unisexual progenies after the 21st day of injection. The incubation period was not the same as observed previously, when *D. nebulosa* was injected with the supernatant from a strain developed from a "sex-ratio" female of *D. willistoni* collected in nature (MALOGOLOWKIN and POULSON 1957). In the control experiment, females from a normal strain of *D. willistoni* were injected with the same supernatant. Incubation period was the same as that reported by MALOGOLOWKIN and POULSON (1957) for *D. willistoni* injected with oöplasm from abnormal eggs of "sex-ratio" females. Results can be seen in Tables 4 and 5 (HSR4).

As indicated above, it is now known that the "sex-ratio" factor is a Treponema, both in the case of *D. willistoni* and in *D. equinoxialis*. However, our previous work and the present experiments seem to indicate that the behavior of the parasite differs in the two cases. In fact: (a) the "sex-ratio" condition can be eliminated by heat treatment in *D. equinoxialis* but not in *D. willistoni*; (b) the transfer from *D. equinoxialis* to *D. nebulosa* cannot be performed directly but can be obtained after a passage in *D. willistoni*; (c) indirect transfer of the "sex-ratio" factor from *D. equinoxialis* to *D. nebulosa* is lost after the third generation (unpublished data).

This shows a complex situation of host-parasite relationships, which must be clarified through further experiments.

SUMMARY

Normal strains of different species of Drosophila were injected with the supernatant from a macerate of "sex-ratio" females from three different species. *D. nebulosa* was successfully infected by direct injection of the "sex-ratio" factor from *D. willistoni*, and by indirect injection of the "sex-ratio" factor from *D. equinoxialis* which was first transferred to *D. willistoni*, from the last named species. However, direct transfer of the sex ratio factor of *D. equinoxialis* into *D. nebulosa* was not obtained. None of the other species were infected by the "sex-ratio" factor from *D. willistoni*, *D. equinoxialis*, or from *D. bifasciata*. The incubation period in *D. nebulosa* is the same as that found in *D. willistoni*, when *D. nebulosa* is injected with the "sex-ratio" factor of *D. willistoni*.

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A GENETIC STUDY OF TWO NEW STRUCTURAL FORMS OF TYROSINASE IN NEUROSPORA[1]

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IN previous work from this laboratory, three apparently unlinked genes concerned with the synthesis of tyrosinase in *Neurospora crassa* have been identified. These genes, designated *T*, *ty-1*, and *ty-2*, respectively, are not functionally equivalent. It was found that whereas the *T* locus has a structure-determining role in the synthesis of the enzyme, the genes *ty-1* and *ty-2* determine whether tyrosinase synthesis is constitutive or inducible but have no influence on the structure of the enzyme actually formed (HOROWITZ, FLING, MACLEOD, and SUEOKA 1960). The question arises whether any other genes of Neurospora, besides the *T* locus, participate in determining the structure of this enzyme. In the present paper, we report the results of a search for such genes.

Owing to the fact that tyrosinase is not essential for the growth of Neurospora, the usual selective procedures cannot be employed for detecting mutations affecting it. (The feasibility of developing a special technique for this purpose is under study.) Because of its nonessential character, however, the enzyme is presumably under weak selection in nature, and it seemed possible that it would show considerable structural variability in wild strains of Neurospora. Such variability had already been indicated by the fact that two different forms of the enzyme were found among a small number of standard wild type stocks in the California Institute of Technology collection (HOROWITZ and FLING 1953). We therefore undertook to examine the tyrosinase from an additional series of wild type strains, collected in various regions of the world. A group of 12 such strains, recently collected in nature, were obtained[3] and their tyrosinases compared with respect to their thermostability and rate of electrophoretic migration on paper. Based on these two criteria, four different forms of the enzyme could be distinguished among the strains. Two of these were indistinguishable from the previously known forms, and two were new types. The genetic relationship of the four forms has been investigated by crosses and heterocaryon studies.

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MATERIALS AND METHODS

Strains: From material sent to the laboratory from various parts of the world, some 25 to 30 cultures of monilioid fungi were established. Purification from bacteria was accomplished by a few serial transfers of aerial hyphae. Each culture was crossed to protoperithecia of both mating types of wild type *Neurospora crassa*, and only those 12 which formed fertile crosses were used for further study. These were grown, and their tyrosinase was extracted, purified, and examined with respect to thermostability and electrophoretic migration. Several of the strains produced too little of the enzyme for characterization, and these were not studied further. Among the remainder, four different tyrosinases were found, as stated above, two of which were indistinguishable from types S and L, known from previous studies (HOROWITZ and FLING 1953), and two new types. The latter were designated Puerto Rico-15 (or PR-15) and Singapore-2 (or Sing-2), respectively, from the names of the strains in which they were discovered. The Puerto Rican strain was isolated from a sample of sugarcane bagasse provided by DR. TEAS and the Singapore strain from some dead bark sent by MR. THOMPSON. Both strains could be crossed with laboratory stocks of *N. crassa*, but fertility was not complete, as indicated by the fact that only random ascospores could be recovered from the crosses. Full fertility was attained by two or three generations of backcrossing to laboratory stocks, selecting for the backcrosses spores which carried one or the other of the new tyrosinase types. These crosses had the further effect of resolving whatever heterocaryotic mixtures may have been present in the natural strains. Evidence was obtained suggesting that the PR-15 strain was heterocaryotic for two different tyrosinases (see Discussion).

To avoid confusion, the designations PR-15 and Sing-2 will be retained for the derivative stocks obtained by backcrossing and for the characteristic forms of tyrosinase produced by them.

Experimental procedures: Crosses were carried out on synthetic medium (WESTERGAARD and MITCHELL 1947). Cultures for tyrosinase assay were grown at 25°C for four days on one of the low-sulfur minimal media described previously (HOROWITZ *et al.* 1960). Assays were performed colorimetrically at 30°C and *p*H 6, with 3,4-dihydroxy-DL-phenylalanine as substrate. Both crude and partially purified (HOROWITZ and FLING 1953) extracts were used as the source of enzyme. Thermal inactivation tests were done at 59° ± 0.02°C. For electrophoresis, a Spinco Model R Paper Electrophoresis Cell was used, with conditions as described in Table 1 and Figure 2. Further details of the electrophoretic procedure are given in an earlier publication (HOROWITZ *et al.* 1960).

RESULTS

The four forms of tyrosinase

Thermostability: Three different levels of thermostability were found among the four tyrosinases. Expressed as half-lives at 59°C, the thermostabilities are approximately five minutes for the L-form of the enzyme, 20 minutes for PR-15, and 70 minutes for the S and Sing-2 forms in 0.1 M sodium phosphate buffer at

*p*H 6. Thermal inactivation data are plotted in Figure 1. The previously reported value of three to four minutes for the L form (HOROWITZ and FLING 1953) was obtained in sodium-potassium phosphate buffer; the value is slightly higher in sodium phosphate (GEST and HOROWITZ 1958). The S and Sing-2 forms show very similar inactivation kinetics over the range 57°–67°C (SUEOKA 1959). We have recently isolated tyrosinase S in pure form (unpublished work from this laboratory), and have determined its thermostability. The half-life of the pure enzyme at 59° is about 65 minutes, in good agreement with the value obtained with partially purified preparations.

Thermostability measurements provide a convenient means for detecting structural differences between enzymes, but their usefulness is limited to cases where the thermostability difference is large, owing to the fact that the stability of proteins is strongly influenced by the composition of the medium. Thus, the rate of thermal inactivation of tyrosinase is affected by traces of substrate (HOROWITZ and FLING 1956), by the ionic environment (GEST and HOROWITZ 1958), and by the presence of other proteins in the solution. Impure preparations of the enzyme contain many substances capable of influencing its stability in one direction or the other, and the net effect of these substances will vary from one preparation to the next, depending on the composition of the initial, crude extract

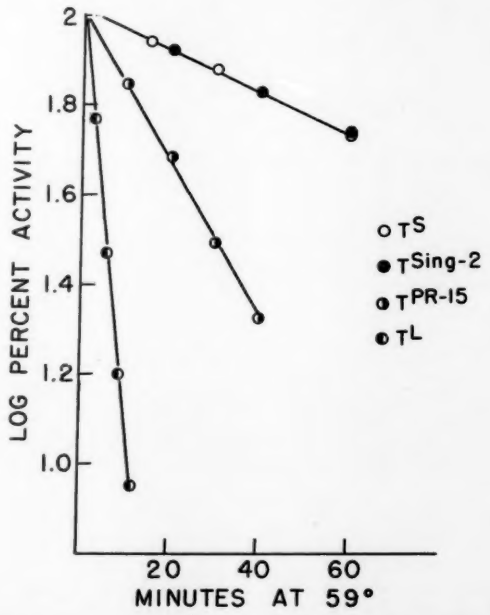

FIGURE 1.—Thermal inactivation of the four tyrosinases of *N. crassa* in 0.1 M sodium phosphate, *p*H 6.

and on the extent of purification. These effects can be minimized and often eliminated by carrying out thermal inactivation measurements with highly diluted samples of the enzyme preparation (HOROWITZ and FLING 1953), but some variability remains, particularly in the case of the most stable forms, S and Sing-2. Working with Sing-2, SUEOKA (1959) found occasional preparations whose half-lives at 59° varied by a factor of ±2 from the modal value of 70 minutes. It was thought at one time that a fifth form of tyrosinase, with a half-life of 83 minutes at 59°C, existed in our stocks (HOROWITZ and FLING 1957), but further work showed it to be indistinguishable from the known S-form by thermostability, electrophoresis, and genetic tests.

Electrophoresis: All four tyrosinases migrate toward the cathode in paper electrophoresis at *p*H 6. The L form is the fastest, S is intermediate, while Sing-2 and PR-15 are the slowest and are electrophoretically indistinguishable from each other. Figure 2 shows electrophoretic patterns of the individual enzymes and of two pairwise mixtures. All pairwise mixtures except Sing-2 + PR-15 can be separated electrophoretically; this indicates that the electrophoretically distinguishable types are structurally different. Table 1 summarizes the electrophoretic and thermostability characteristics which form the basis for recognition of the four enzyme types. The electromigration rates given in the table are observed rates, uncorrected for electroosmotic flow of the buffer.

Unlike the thermal inactivation rates, the rates of electrophoretic migration of the enzymes are, in our experience, independent of the purity of the preparations. The same values are found for crude extracts as for highly purified preparations. Occasionally, however, crude preparations of S and Sing-2 show one or two secondary bands of tyrosinase activity in addition to the principal

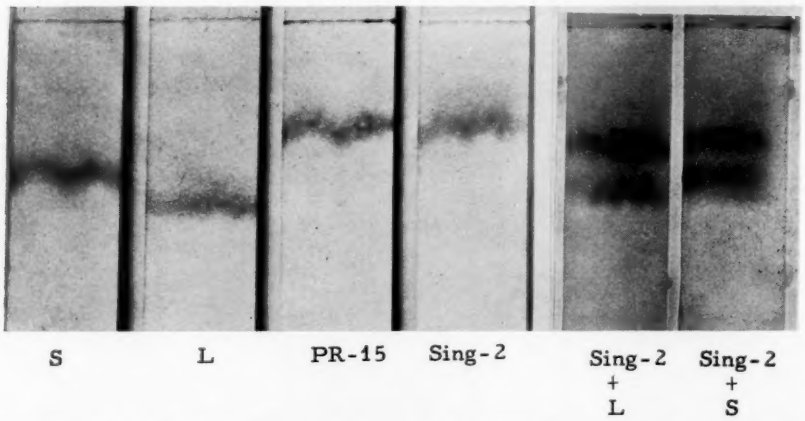

FIGURE 2.—Paper electrophoresis (20 hours) of the four tyrosinases and of two pairwise mixtures. The horizontal line at the top of each strip marks the point of application of the enzyme solutions. Migration is toward the cathode. Conditions as stated in Table 1. The enzymes were visualized by spraying the strips with a solution of 3,4-dihydroxy-DL-phenylalanine.

TABLE 1

Properties of the four tyrosinase types

Type	Thermostability* (half-lives at 59°, min.)	Electrophoretic migration† (mm per hour, on paper)
S	70	2.0
L	5	2.25
PR-15	20	1.5
Sing-2	70	1.5

* In 0.1 M sodium phosphate buffer, *p*H 6.
† In 0.05 M sodium phosphate buffer, *p*H 6, containing 0.1 percent bovine serum albumin. Current, 1.25 ma per paper strip in a Spinco Model R Paper Electrophoresis Cell. The rates are not corrected for electroosmotic flow of the buffer.

band. The secondary bands migrate more slowly than the principal band. The components giving rise to the extra bands are extremely labile. Storage of the preparations overnight in the refrigerator, freezing and thawing, or brief warming causes them to disappear. Apparently the same phenomenon has been observed by Fox, Burnett and Fuchs (1959). These authors drew the conclusion that the labile components are normal precursors of tyrosinase and proposed that in the terminal stages of tyrosinase synthesis a thermolabile form of the enzyme (which they suggest may be identical with our L tyrosinase) is converted into a thermostable form (corresponding to our S). This interpretation is opposed by considerable evidence. In our experiments, the secondary bands do not migrate at the same rate as the L form of the enzyme, but slower. Furthermore, we have shown that tyrosinase S and L form stable mixtures both *in vitro* and *in vivo* (i.e, in mycelia heterocaryotic for the S and L alleles); if these mixtures are heated, the loss of tyrosinase activity precisely follows the course predicted for simple first-order inactivation of both forms (Horowitz and Fling 1956). A more reasonable interpretation of the secondary bands would seem to be that they result from interactions between the enzyme and other components of the crude extracts, or between tyrosinase molecules, giving rise to unstable associations. The subject clearly requires more study before conclusions can be drawn.

Genetics of the tyrosinases

Results of crosses: Two series of crosses were carried out among the four strains. In Series A, the six possible nonreciprocal crosses were made, and complete tetrads were analyzed for segregation of tyrosinase types. In Series B, the crosses L × S, L × Sing-2, and L × PR-15 were made, and for reasons explained below, asci containing one unripe and three ripe spore pairs were selected for analysis. Cultures were grown from the ascospores, the enzyme was extracted and its thermostability and/or electrophoretic migration measured. In most cases, crude extracts could be used for these tests; partial purification of extracts was carried out where necessary to check results which appeared ambiguous or unusual.

Table 2 summarizes the results of the crosses. Simple Mendelian segregation of the enzymatic characters was observed in all cases. No recombination was detected. Recombination between the structurally dissimilar tyrosinases could be manifested in any of the following ways: (a) both recombinants might yield

TABLE 2

Summary of genetic data on the tyrosinase types

Cross: X×Y Series	S×L* A†	S×L* B‡	PR-15×L A§	PR-15×L B‡	Sing-2×L A§	Sing-2×L B‡	S×PR-15 A§	S×Sing-2 A‡	PR-15×Sing-2 A‖	Totals
Complete tetrads	73		21		30		31	23	18	196
M-I segregations	31		7		8		16	6	4	72
M-II segregations	42		14		22		15	17	14	124
Ratio X:Y	146:146		42:42		60:60		62:62	46:46	36:36	
¾-tetrads	7	7	8	13	10		16	..	..	61
M-I segregations	3	3	1	3	5		5	..	..	20
M-II segregations	4	4	7	10	5		11	..	..	41
Ratio X:Y	14:7	13:8	9:15	18:21	14:16		29:19			
½-tetrads	2	12	9	3	8		2	..	..	36
Ratio X:Y	2:2	8:16	8:10	3:3	10:6		3:1			
¼-tetrads	2	5	4	5	3		37	..	..	56
Ratio X:Y	2:0	3:2	4:0	2:3	2:1		26:11			
Total X:Y	188:181		86:94		86:83		120:93	46:46	36:36	
Percent M-II segregations	57.5		73.8		67.5		55.3	73.9	77.8	
Upper limit on recombination frequency	0.014		0.026		0.030		0.022	0.061	0.077	

* Includes previously published data on this cross (HOROWITZ and FLING 1953).
† Both thermostability and electrophoresis tests on 22 complete tetrads.
‡ Electrophoresis tests only.
§ Both thermostability and electrophoresis tests on all progeny.
‖ Thermostability tests only.

active new forms of the enzyme, (b) one recombinant might yield an active new form, the other an inactive form, (c) both recombinants might yield inactive forms. Since tyrosinase is presumably responsible for the melanization that accompanies the maturation of ascospores (SCHAEFFER 1953), tyrosinase-deficient recombinants might fail to mature and, if so, would not be detected. This would not be the case if melanization were not essential for ascospore germination, or if the tyrosinase of ascospores were entirely of maternal origin, but no conclusive evidence exists on either of these points. It is clear from these considerations that analysis of tetrads containing four ripe spore pairs can, in principle, detect outcome (a) with certainty, but (b) and (c) only doubtfully. Analysis of tetrads containing one unripe spore pair would detect (b), however, and this was the reason for the Series B crosses. As yet, there is no certain method for detecting (c). If recombination resulted in the abortion of one or both recombinant spore pairs, however, an increase in the frequency of incomplete asci over the number normally present would be observable. We have not made a statistical study of this point, but the frequency of unripe spores was not noticeably abnormal in these crosses.

Calculation of upper limits on the recombination frequencies: We wish to compute here the maximum recombination frequencies allowed by the data of Table 2. The lower limits are, of course, zero in all cases, since no recombinants were in fact found. The following discussion is based in part on a previous note (HOROWITZ 1953).

We shall consider first the crosses of Series B. If d is the recombination frequency between two linked genes, the frequency of recombinant tetrads will be $2d$, since each such tetrad contains two recombinant and two nonrecombinant spore pairs. The frequency of nonrecombinant tetrads is $1 - 2d$. If n tetrads are analyzed, the probability that none will show recombination is then $(1 - 2d)^n$. This expression is valid also for ¾-tetrads.

Assume that one recombinant type fails to mature and that a number of asci with one immature spore pair are analyzed, as in Series B. Owing to accidents of development, not all ascospores germinate, so that, in addition to ¾-tetrads, some ½- and ¼-tetrads will result. If the numbers of these are, respectively, n', p', and q', then the probabilities $P'(0)$ that no recombination will be found are:

$$P'(0)_{3/4\text{-tetrads}} = (1 - 2d)^{n'}$$
$$P'(0)_{1/2\text{-tetrads}} = (1 - \tfrac{2}{3} \times 2d)^{p'}$$
$$P'(0)_{1/4\text{-tetrads}} = (1 - \tfrac{1}{3} \times 2d)^{q'}$$

and the probability that no recombinants will be found in the sample as a whole is

$$P'(0) = (1 - 2d)^{n'}(1 - 4/3d)^{p'}(1 - \tfrac{2}{3}d)^{q'}. \qquad (1)$$

By letting $d = 0.01$ in equation (1) and solving for $P'(0)$, the probability that no recombinants will be found if the segregating characters are one map unit apart is obtained. When this calculation is performed for the three crosses of Series B, it is found that the $P'(0)$'s are all less than four percent. (In applying the equation to Series B, cognizance is taken of the fact that ¾-tetrads of the type selected make up not over ten percent of all tetrads by multiplying the observed n', p', and q' by 10). In view of the smallness of the chance that recombination would have been missed in these crosses, even assuming close linkage, it is unlikely that the ¾-tetrads contain a larger proportion of crossovers than other tetrads. The Series B tetrads can therefore be lumped with those of Series A for calculating upper limits on the recombination frequencies.

If both recombinants are viable and recognizable (outcome (a) above), then the probability $P(0)$ of no recombinants in a random sample of asci is, by reasoning analogous to that above,

$$P(0) = (1 - 2d)^n(1 - 5/3d)^p(1 - d)^q \qquad (2)$$

where n = the number of complete + ¾-tetrads, p = the number of ½-tetrads, and q = the number of ¼-tetrads analyzed. If $P(0) = 0.05$, then d defines a map interval within which the probability is 0.95 that one or more crossovers would have occurred in a sample of the given size. This value of d is taken as the upper limit on the recombination frequency. In solving for d, the following approximation to equation (2) is useful where p and q are not equal to zero:

$$P(0) = e^{-2nd - 5/3pd - qd}. \qquad (2')$$

Upper limits obtained by equations (2) or (2′) are listed in Table 2.

It is evident from the data of Table 2 that the thermostability and electrophoretic properties of the tyrosinase molecule are determined by a small chromosomal region. We refer to this region as the T locus and, in the absence of contradictory evidence, assume that the four dissimilar tyrosinases represent four

alleles: T^S, T^L, $T^{PR\text{-}15}$, and $T^{Sing\text{-}2}$. The T locus is in the right arm of linkage group I (HOROWITZ and FLING 1956).

Heterocaryon experiments: In a previous report, it was shown from the kinetics of thermal inactivation that the tyrosinase produced by a heterocaryon between T^L and T^S is a mixture of the L and S forms (HOROWITZ and FLING 1956). This conclusion has been confirmed in the present experiments by paper electrophoresis, by means of which the enzyme from the $(T^L)(T^S)$ heterocaryon can be separated into two zones corresponding to the L and S forms. When an extract of the heterocaryon was heated at 60°C for ten minutes before electrophoresis, only one zone of activity appeared on the paper strips, and this in the position known to be occupied by the S form.

Similar results have been obtained with heterocaryons $(T^L)(T^{Sing\text{-}2})$ and $(T^L)(T^{PR\text{-}15})$. As in the $(T^L)(T^S)$ case, these heterocaryons were balanced with the biochemical markers *hist-3* and *ad-3*. Again, mixtures of the two corresponding tyrosinases were formed by the heterocaryons, with no evidence of dominance or interaction. Thermal inactivation curves for the two new heterocaryons are shown in Figure 3. These results conform with what is expected on the assumption that the four tyrosinases are determined by a series of allelic genes.

DISCUSSION

The most remarkable feature of the enzyme revealed by these studies is its

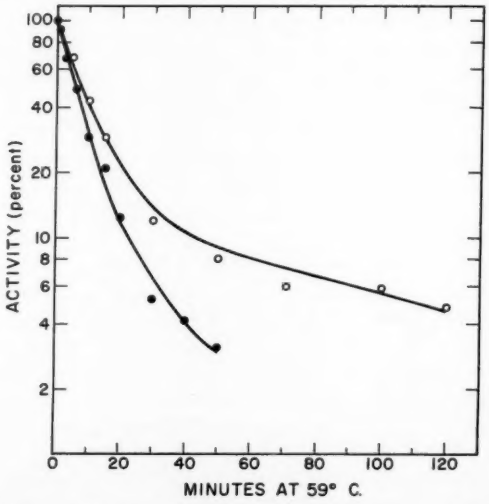

FIGURE 3.—Thermal inactivation of tyrosinases from heterocaryons. Open circles, Het (Sing-2)(L). Solid circles, Het (PR-15)(L). Conditions as in Figure 1. Points are experimental values; curves are theoretical for the following mixtures: open circles, 14% of half-life 75 minutes + 86% of half-life six minutes; solid circles, 15% of half-life 21 minutes + 85% of half-life five minutes.

high degree of structural variability in nature. This variability, or polymorphism, is so extensive that it is impossible to single out a wild type form of the enzyme. The four different forms were found in about a dozen wild strains of Neurospora by the use of two tests—electrophoresis and thermostability. It is reasonable to suppose that by applying other sensitive criteria, additional forms of tyrosinase could be found. Although the strains we have used originated for the most part in widely separated geographical regions, we have evidence for interclonal variation within a single region. The sample of sugarcane bagasse from which PR-15 was obtained also yielded an isolate which produced the S form. Judging from its thermal inactivation curve, the original PR-15 strain itself appears to have been a heterocaryon between $T^{PR\text{-}15}$ and T^{L}. The T^{L} component was not recovered in the first generation progeny, however. This structural variability is possibly due to the weakness of the selection that operates on tyrosinase, a nonessential enzyme, in nature. No comparable study has been made on any essential enzyme of Neurospora, however.

Genetically, this study has found evidence for only one structure-determining genetic region for the enzyme. The fact that selective methods cannot be used for detecting recombinants among them limits the possibilities for subdivision of this region by further recombination studies on the four tyrosinases. The heterocaryon evidence, however, suggests that the differences observed among the four enzymes are determind by a functionally unitary region. It should be possible to attain further resolution of the *T* locus by the use of tyrosinase-deficient mutants. At the present time, two such mutants—*ty-1* and *ty-2*—are known, but neither of them is an allele of the *T* locus. These mutants have no effect on the structure of the enzyme, but control its induction (Horowitz *et al.* 1960).

It is instructive to compare our results with those of Markert (1950) on the genetics of tyrosinase in *Glomerella cingulata*. Markert studied a number of mutants in which the amount of tyrosinase activity ranged from a few percent of normal to ten or more times the normal activity, and he showed that these effects were produced by a number of different genes. It could not be determined from these results, however, which, if any, of the various genes had a structure-determining influence on the enzyme and which had merely quantitative effects. In a subsequent study, Markert and Owen (1954) attempted, by serological means, to detect structural alterations in the tyrosinase of the mutants, but without success. Our study agrees with the foregoing in indicating multigenic control of tyrosinase synthesis, but it clearly points to one locus as the structure-determining region.

The ease with which it is possible, by analysis of thermal inactivation curves, to obtain quantitative estimates of the amounts of two different tyrosinases in heterocaryons suggests that this system would be useful for investigating the effect of varying the nuclear ratio on the amounts of the two enzymes formed. Some data bearing on this question have been obtained (Sueoka 1959). They indicate a rather close parallelism between the nuclear ratio and the ratio of the

corresponding enzyme activities; but whether or not the different tyrosinases have the same specific or molar activities is not yet known.

SUMMARY

The tyrosinase of *Neurospora crassa* shows a remarkable degree of structural variability in nature. Four different forms of the enzyme have been found in a small number of newly collected wild strains. Two of these were indistinguishable from the previously known forms of the enzyme, S and L. The other two are new types, called Puerto Rico-15 (PR-15) and Singapore-2 (Sing-2). The four enzymes differ in their thermostability and/or electrophoretic properties. Simple Mendelian inheritance of the enzymatic differences was observed. No recombination was detected among the different forms, nor was dominance or interaction of any kind found in heterocaryons. It is concluded that the structural differences are determined by a small, functionally unitary genetic region, the *T* locus. The four tyrosinases appear to represent four different alleles at this locus.

ACKNOWLEDGMENT

The assistance of MR. STEVE HEINEMAN during the course of these experiments is gratefully acknowledged.

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THE GENETICS OF SEXUAL ISOLATION IN DROSOPHILA PAULISTORUM[1]

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SEXUAL isolation is a "weakness or lack of mutual attraction between males and females of different species" (DOBZHANSKY 1951). Occurrence of rudiments of sexual isolation within a species, between races or subspecies, may indicate that a process of speciation, of splitting of a single species into two or several derived ones, is under way. It is, indeed, to be expected on theoretical grounds that genetic variability from which reproductive isolating mechanisms could be built should be found also intraspecifically.

The genetics of sexual isolation between two morphologically similar, sibling, species, *Drosophila pseudoobscura* and *Drosophila persimilis*, has been analyzed by TAN (1946). Factors distinguishing the mating behavior of *D. persimilis* from that of *D. pseudoobscura* were found in at least the X and the second chromosomes. The present article describes a series of experiments directed, for the first time, toward the study of the genetics of sexual isolation, i.e., of isolation in the process of formation, between subspecies.

Drosophila paulistorum Dobzhansky and Pavan is one of the five sibling species which constitute the *willistoni* group of sibling species. The morphological characteristics and the relationships of this species to *D. tropicalis*, *D. willistoni*, and *D. equinoxialis* were analyzed by BURLA *et al.* (1949). *D. tropicalis* males were found to inseminate some *D. paulistorum* females and *D. paulistorum* males occasionally copulated successfully with females of the other three species. However, these interspecific inseminations never resulted in the production of hybrid progenies. Later, DOBZHANSKY, EHRMAN and PAVLOVSKY (1957) analyzed the relationship between *D. paulistorum* and *D. insularis*, the last *willistoni* sibling species to be described. Here hybrid progenies, although wholly sterile, were produced in some instances by heterogamic insemination. Without doubt, the five sibling species are completely isolated reproductively.

Extensive collections of *D. paulistorum* in 1957 and 1958 from localities in Central and South America yielded a group of geographic strains which, when brought into the laboratory and intercrossed, gave varied and surprising results. Not all the strains could be outcrossed with equal ease; some matings produced no hybrids at all, while others yielded fertile female hybrids and F_1 males that were completely sterile (DOBZHANSKY and SPASSKY 1959). Eventually, six groups of geographic populations were distinguished:

[1] This investigation was supported by a postdoctoral fellowship, GF-9033, from the Division of General Medical Sciences, U.S. Public Health Service.

1. *Centro-American* group, from Guatemala to Costa Rica.
2. *Amazonian* group, from Panama through northern Brazil.
3. *Andean-South Brazilian* group, from southern Colombia, Ecuador, Peru, Bolivia to southern Brazil.
4. *Orinocan* group, from Panama and eastern Colombia to Venezuela and British Guiana.
5. *Guianan* group, from Georgetown, in British Guiana (a single strain).
6. *Transitional* group, from several localities in Colombia.

Crosses between the first three subspecies always produce fertile female and sterile male hybrids; this male sterility is genic in nature, and expresses itself via the genotype of hybrid mothers (EHRMAN 1960a). The male offspring of an intersubspecific hybrid female is sterile regardless of its own genotype and that of its male parent; the mother needs, however, be "hybrid" only with respect to any one chromosome in the diploid complement of three pairs to produce this remarkable phenomenon.

The purpose of the present work is to analyze three of the subspecies, the Centro-American, the Amazonian, and the Andean-South Brazilian, with regard to the genetic basis of another reproductive isolating mechanism, namely the sexual isolation between them.

MATERIALS AND METHODS

The plan followed in ascertaining the role of each of the two autosomes and of the X chromosome in the genetic architecture of the sexual isolation barrier via a mating preference technique was the same as the one employed to analyze the genetics of the sterility of the hybrid males (see EHRMAN 1960a for details). The object was to transfer one marked chromosome into the nuclear and cytoplasmic background of an alien subspecies. At no time could two marked chromosomes be transferred across the subspecific barrier simultaneously. *D. paulistorum* is a species delicate enough so that, at least in laboratory cultures, flies carrying several mutant genes are apt to have a viability too low to permit carrying out experiments on their mating preferences successfully.

Because of the difficulty of obtaining progeny from intersubspecific matings, the hybrid offspring necessarily come from a limited number of females. The initial intersubspecific crosses were therefore made in a direction which minimized the sexual isolation barrier. Thus, the F_1 hybrids shown in Table 1 were obtained by crossing Centro-American ♀♀ × Amazonian ♂♂; Tables 2, 3, 4 = Amazonian ♀♀ × Centro-American ♂♂; Tables 5, 6, 7, 8 = Centro-American ♀♀ × Andean-South Brazilian ♂♂; Table 9 = Andean-South Brazilian ♀♀ × Amazonian ♂♂.

Backcross progenies were obtained by crossing F_1 hybrid females carrying suitable marker genes in certain chromosomes to males of one or the other of the parental subspecies. The backcrosses were repeated in each of three successive generations, always selecting as female parents the carriers of subspecies-foreign

chromosomes. For each combination of parental subspecies, two series of recurrent backcrosses were made, to each of these subspecies. The F_1 hybrid females between subspecies A and B obviously contain one A and one B chromosome in each pair; the F_1 hybrid males have the X of their mother, and the Y of their father, and an A and a B autosome of each parent. In the backcrosses of the A/B hybrid females to A males, the B chromosomes, except the one with the gene marker, tend to be gradually replaced because only the one with the gene marker is selected for; in the backcrosses to B males, the A chromosomes tend to be eliminated. In the progeny of the third backcross, most of the flies will carry chromosomes of one subspecies only, except the "foreign" chromosome with the genetic marker (and sometimes also a foreign Y chromosome).

The "control" crosses involved the use of sisters of the same females which were used in the experimental series, but not containing the foreign chromosomes with the genetic markers. The progenies were, consequently, like the experimental ones, except that they did not contain the foreign marked chromosome.

In most instances the foreign chromosome contained only a single mutant gene which served as a marker. This was nevertheless deemed a satisfactory experimental technique, for two reasons: First, whenever more than one marker was present, crossing over was found to be suppressed in the intersubspecific crosses. Second, as shown by the unpublished data of DOBZHANSKY and PAVLOVSKY, the subspecies involved in these experiments differed in at least one inversion in each of their five chromosome "arms" (the X chromosome and second chromosome in *D. paulistorum* are metacentric). This does not, of course, exclude the possibility that some undetected crossing over occurs in the hybrids, but such crossing over will not be frequent. This makes using a single gene marker per chromosome a legitimate procedure; more than one could be used in some crosses, but this would have reduced the viability of the flies to a greater extent than would be desirable.

Flies to be tested for mating preferences were aged from four days to one week in isolation from flies of the opposite sex. They were then placed, without etherization, in glass observation chambers; small Petri dishes containing a small amount of food were used for this purpose.

Generally, the ratio of the sexes put into an observation chamber at one time was three hybrid females plus three males of one parental subspecies and three males of the other parental subspecies. Or, where hybrid males were to be tested, three hybrid males plus three females of one parental subspecies and three females of the other parental subspecies were used. These numbers were sometimes changed because of a shortage of virgin females. In all, 4871 virgin females were used in the course of these experiments, and *D. paulistorum* virgins must be collected every three to four hours. Observation chambers testing hybrid females were watched until approximately 20 copulae were recorded. Observation chambers testing hybrid males were watched until 20 copulae involving them were recorded.

A pictorial study of both the material and the method, and of the mating behavior in *D. paulistorum* has been published by EHRMAN and STRICKBERGER (1960).

Hybrids between the Centro-American and Amazonian races: Females of the Centro-American subspecies heterozygous for the second chromosome dominant gene Spot (a dark spot on the thorax) were crossed to the Amazonian subspecies males heterozygous for the second chromosome dominant marker Plum (an eye color). Among the F_1 hybrids, Spot/Plum females were selected to be the progenitors of the backcross progenies. Spot, Plum, and wild-type hybrid females and males were used for tests on their mating preferences. The females were placed together with equal numbers of wild-type males from the Honduras strain of the Centro-American subspecies, and from the Belem strain of the Amazonian subspecies. As shown in Table 1, the 22 copulae observed were all with Centro-American males. The males were given equal numbers of virgin Honduras females and Belem females. Of the 19 copulae observed, 17 were with Honduras and two with Belem males.

The Spot/Plum F_1 females were backcrossed to wild-type males of both parental subspecies. In two following generations, Spot or Plum females were backcrossed to wild-type males of the opposite subspecies. In every generation, females and males carrying the mutant markers were tested for their mating preferences by placing them together with equal numbers of wild-type individuals of the

TABLE 1

Copulae observed in crosses between Centro-American (CA) females and Amazonian (Am) males strains. The second chromosomes marked by Spot (CA) and Plum (Am) mutations

	Number	Copulae with CA	Copulae with Am	Number of hybrids tested	Chi-square	P
			Tests of hybrid females			
F_1	22	22	0	72	20.04	<0.01
			Backcrosses to Centro-American parent			
BC_1	20	10	10	27	....	...
BC_2	20	19	1	25	14.45	<0.01
BC_3	20	19	1	24	14.45	<0.01
			Backcrosses to Amazonian parent			
BC_1	20	4	16	34	6.05	0.02–0.01
BC_2	22	5	17	51	5.50	0.02–0.01
BC_3	22	2	20	30	13.14	<0.01
			Tests of hybrid males			
F_1	19	17	2	48	10.32	<0.01
			Backcrosses to Centro-American parents			
BC_1	20	17	3	58	8.45	<0.01
BC_2	20	19	1	36	14.45	<0.01
BC_3	22	20	2	38	13.14	<0.01
			Backcrosses to Amazonian parent			
BC_1	20	0	20	38	18.05	<0.01
BC_2	19	0	19	51	17.04	<0.01
BC_3	20	0	20	38	18.05	<0.01

opposite sex of the parental subspecies. All the results appear in Table 1. In the backcrosses to the Centro-American subspecies, the hybrids showed a decided preference for Centro-American partners (except for the BC_1 females which appeared to be neutral). In the backcrosses to the Amazonian subspecies, the preference for the Amazonian partners rapidly becomes emphatic.

To test the possibility that the source of the cytoplasm may be of importance, the same crosses as above were repeated starting with Plum Amazonian females and Spot Centro-American males. The results are reported in Table 2. Comparison with the data in Table 1 discloses an interesting situation. The F_1 hybrid females now prefer males of the Amazonian subspecies (16 out of the 20 copulations observed). This is also true of the F_1 hybrid males (17 of the 20 copulations with the Amazonian females). Effects on the sexual preference of the maternal cytoplasm or of the foreign X chromosomes are therefore indicated. These are, however, not borne out in other experiments (see below).

In any case, this maternal effect does not extend beyond the first generation of hybrids. Comparison of the data in Tables 1 and 2 show that the sexual preferences of all the backcross hybrids were similar or identical in the reciprocal crosses.

To test the effects on the sexual preferences of the foreign third chromosomes, Amazonian females heterozygous for the dominant marker Delta (wing venation) in the third chromosome were crossed to Centro-American males which

TABLE 2

Copulae observed in crosses between Amazonian (Am) females strains and Centro-American (CA) males strains. The second chromosome marked by Spot (CA) and Plum (Am) mutations A Reciprocal Cross

	Number	Copulae with CA	Copulae with Am	Number of hybrids tested	Chi-square	P
			Tests of hybrid females			
F_1	20	4	16	163	6.05	0.02–0.01
			Backcrosses to Centro-American parent			
BC_1	22	18	4	39	7.68	<0.01
BC_2	21	20	1	26	17.04	<0.01
BC_3	20	20	0	46	18.05	<0.01
			Backcrosses to Amazonian parent			
BC_1	20	1	19	68	14.45	<0.01
BC_2	20	0	20	41	18.05	<0.01
BC_3	20	0	20	26	18.05	<0.01
			Tests of hybrid males			
F_1	20	3	17	61	8.45	<0.01
			Backcrosses to Centro-American parent			
BC_1	20	11	9	31	0.05	0.90–0.80
BC_2	20	17	3	30	8.45	<0.01
BC_3	20	19	1	31	14.45	<0.01
			Backcrosses to Amazonian parent			
BC_1	20	2	18	33	11.25	<0.01
BC_2	20	2	18	33	11.25	<0.01
BC_3	20	1	19	27	14.45	<0.01

carried on one of their third chromosomes the dominant Minute (bristles) and the recessive ebony (body color). In the F_1 generation, the Delta/Minute-ebony females were used as progenitors of the backcross progenies, and their sibs were used for tests of the mating preferences of the F_1 hybrids (ebony is approximately 50 crossover units from Minute, and is used here as a check on the suppression of crossing over in the hybrids). The results are reported in Table 3. The F_1 hybrid females accepted Centro-American males, while the F_1 hybrid males appeared to be neutral (although the F_1 hybrid males shown in Table 2 were accepted preferentially by the Amazonian females, this difference in behavior is definitely not statistically significant).

The backcross progenies showed that the sexual preference of the hybrids were for the subspecies of the recurrent parent. It appears that the third chromosome, like the second chromosome, does not by itself determine the sexual preferences in these crosses.

To test the effects of the X chromosome, Amazonian females homozygous for the sex-linked gene for white eyes were crossed to wild-type Centro-American males from the Honduras strain (no gene markers are available in the X chromosome of the Centro-American subspecies). The gene white in *D. paulistorum* is incompletely recessive, heterozygous females being identifiable by their dilute red eye color. The tests of the sexual preferences of the F_1 hybrids are reported in Table 4. The hybrid females preferred Centro-American males despite their

TABLE 3

Copulae observed in crosses between Amazonian (Am) females and Centro-American (CA) males strains. The third chromosome marked by Minute (CA), ebony (CA), and Delta (Am) mutations

	Number	Copulae with CA	Copulae with Am	Number of hybrids tested	Chi-square	P
			Tests of hybrid females			
F_1	19	17	2	77	10.32	<0.01
			Backcrosses to Centro-American parent			
BC_1	20	18	2	37	11.25	<0.01
BC_2	20	19	1	19	14.45	<0.01
BC_3	20	19	1	44	14.45	<0.01
			Backcrosses to Amazonian parent			
BC_1	20	2	18	52	11.25	<0.01
BC_2	20	1	19	50	14.45	<0.01
BC_3	20	1	19	49	14.45	<0.01
			Tests of hybrid males			
F_1	20	8	12	123	0.45	0.70–0.50
			Backcrosses to Centro-American parent			
BC_1	20	14	6	35	2.45	0.20–0.10
BC_2	20	19	1	40	14.45	<0.01
BC_3	20	19	1	35	14.45	<0.01
			Backcrosses to Amazonian parent			
BC_1	20	2	18	45	11.25	<0.01
BC_2	20	2	18	72	11.25	<0.01
BC_3	20	2	18	60	11.25	<0.01

TABLE 4

Copulae observed in crosses between Amazonian (Am) females and Centro-American (CA) males strains. The first chromosome marked by a white (Am) mutation

	Number	Copulae with CA	Copulae with Am	Number of hybrids tested	Chi-square	P
			Tests of hybrid females			
F_1	22	19	3	45	10.22	<0.01
			Backcrosses to Amazonian parent			
BC_1	20	0	20	44	18.05	<0.01
BC_2	20	1	19	83	14.45	<0.01
BC_3	20	0	20	37	18.05	<0.01
			Tests of hybrid males			
F_1	20	8	12	99	0.45	0.70–0.50
			Backcrosses to Amazonian parent			
BC_1	20	0	20	46	18.05	<0.01
BC_2	20	0	20	28	18.05	<0.01
BC_3	20	0	20	21	18.05	<0.01

Amazonian cytoplasm. The F_1 hybrid males (having white eyes) were not clearly preferred by females of either subspecies.

The F_1 hybrid females were backcrossed to white-eyed Amazonian males in three successive generations. The results are reported in Table 4. No backcrosses to the Centro-American parent could be made because of the lack of gene markers. Both females and males in the backcross progenies preferred and were preferred by Amazonian partners. Now, the backcross females carried one Amazonian and one Centro-American X chromosome. The backcross males had, however, the Centro-American X chromosome, or at least the part containing the wild-type allele of the gene white. They also had an Amazonian Y chromosome.

It appears then, that in the Amazonian × Centro-American hybrids, sexual preference is decided by which subspecies contributes more than half of the genome, no one chromosome being clearly more important than the others.

Hybrids between the Centro-American and Andean-South Brazilian races: The crosses made to study the sexual preferences of the hybrids carrying various combinations of the chromosomes of the Centro-American and the Andean-South Brazilian subspecies were quite analogous to those described above for the Centro-American × Amazonian hybrids. The X chromosome could not, however, be tested on account of the absence of suitable genetic markers.

Table 5 reports the results of the tests of the second chromosomes. The parents of the crosses were Centro-American females with the gene marker Spot, and Andean-South Brazilian males heterozygous for the dominant second chromosome gene Bubbled (inflated wings). As usual, the F_1 hybrid females carrying the two markers from the two parental subspecies were used as parents of the backcross progenies, and their siblings showing either or neither marker were tested for sexual preferences. As shown in Table 5, the F_1 hybrids of both sexes were neutral between the parental subspecies.

The behavior of the backcrosses is most interesting, and clearly different from

TABLE 5

Copulae observed in crosses between Centro-American (CA) females and Andean-South Brazilian (ASB) males strains. The second chromosome marked by Spot (CA) and Bubbled (ASB) mutations

	Number	Copulae with CA	Copulae with ASB	Number of hybrids tested	Chi-square	P
			Tests of hybrid females			
F_1	20	9	11	24	0.05	0.90–0.80
			Backcrosses to Centro-American parent			
BC_1	21	11	10	24	....	...
BC_2	25	18	7	30	4.00	0.05–0.02
BC_3	20	15	5	29	4.05	0.05–0.02
			Backcrosses to Andean-South Brazilian parent			
BC_1	20	9	11	29	0.05	0.90–0.80
BC_2	20	9	11	28	0.05	0.90–0.80
BC_3	20	10	10	26	....	...
			Tests of hybrid males			
F_1	20	8	12	26	0.45	0.70–0.50
			Backcrosses to Centro-American parent			
BC_1	23	9	14	39	0.69	0.50–0.30
BC_2	20	7	13	46	1.25	0.30–0.20
BC_3	20	6	14	52	2.45	0.20–0.10
			Backcrosses to Andean-South Brazilian parent			
BC_1	22	11	11	36	....	...
BC_2	19	10	9	48	....	...
BC_3	20	9	11	28	0.05	0.90–0.80

what we observed in the Centro-American × Amazonian hybrids described above. In the Centro-American × Andean-South Brazilian hybrids (Table 5), the sexual neutrality is retained as long as the fly carries a subspecies-foreign chromosome. Even in the progeny of the third backcross, when the genotype must have been mostly that of the recurrent parent, no clear sexual preference is apparent.

The above conclusion was tested by a control experiment shown in Table 6. The pure Centro-American females with the gene Spot preferred Centro-American males; the pure South Brazilian males with the gene Bubbled were as clearly preferred by South Brazilian females (Angra strain). In the progenies of the third backcrosses, females and males were selected which did not carry the mutant markers, and therefore presumably were free of subspecies-foreign chromosomes. The test showed they preferred, and were preferred by, individuals of the subspecies to which the recurrent parent belonged.

The tests of the effects of third chromosomes are reported in Table 7. The original cross was of Minute-ebony Centro-American females to Delta Andean-South Brazilian males. The F_1 hybrid females and males were neutral in sexual preference between the parental subspecies. This agrees with what was observed in the tests of the second chromosomes (see above). The behavior of the backcross progenies was different: the females perhaps showed a slight preference for males

TABLE 6

Copulae observed in crosses between Centro-American (CA) females and Andean-South Brazilian (ASB) males strains. The second chromosome marked by Spot (CA) and Bubbled (ASB) mutations
Control

	Number	Copulae with CA	Copulae with ASB	Number of hybrids tested	Chi-square	P
		Tests of Spot (Centro-American) females				
P	20	16	4	20	6.05	0.02–0.01
		Backcrosses of Centro-American parent—tests of hybrid females				
BC_3	20	16	4	23	6.05	0.02–0.01
		Backcrosses to Andean-South Brazilian parent				
BC_3	20	4	16	25	6.05	0.02–0.01
		Tests of Bubbled (South Brazilian) males				
P	20	4	16	20	6.05	0.02–0.01
		Backcrosses to Centro-American parent—tests of hybrid males				
BC_3	20	16	4	20	6.05	0.02–0.01
		Backcrosses to Andean-South Brazilian parent				
BC_3	20	3	17	24	8.45	<0.01

TABLE 7

Copulae observed in crosses between Centro-American (CA) females and Andean-South Brazilian (ASB) males strains. The third chromosome marked by Minute (CA), ebony (CA), and Delta (ASB) mutations

	Number	Copulae with CA	Copulae with ASB	Number of hybrids tested	Chi-square	P
		Tests of hybrid females				
F_1	20	9	11	25	0.05	0.90–0.80
		Backcrosses to Centro-American parent				
BC_1	22	13	9	37	0.41	0.70–0.50
BC_2	20	12	8	25	0.45	0.70–0.50
BC_3	20	12	8	33	0.45	0.70–0.50
		Backcrosses to Andean-South Brazilian parent				
BC_1	20	9	11	29	0.05	0.90–0.80
BC_2	22	9	13	37	0.41	0.70–0.50
BC_3	20	8	12	38	0.45	0.70–0.50
		Tests of hybrid males				
F_1	20	15	5	39	4.05	0.05–0.02
		Backcrosses to Centro-American parent				
BC_1	20	18	2	42	11.25	<0.01
BC_2	20	18	2	41	11.25	<0.01
BC_3	20	18	2	44	11.25	<0.01
		Backcrosses to Andean-South Brazilian parent				
BC_1	20	11	9	38	0.05	0.90–0.80
BC_2	20	3	17	38	8.45	<0.01
BC_3	20	3	17	54	8.45	<0.01

of the subspecies of the recurrent parent, but in no case did their preference reach the level of statistical significance. On the other hand, the backcross males

have become gradually shifted towards being preferred by the females of the subspecies of the recurrent parent. The F_1 hybrid males, and the males from the first backcross to the South Brazilian parent, are neutral or slightly preferred by the Centro-American females. In the second and third backcrosses the preference by the females for the recurrent parental subspecies becomes obviously significant.

To make sure that the intrepretation of the data reported in Table 7 was valid, a control experiment was run, and reported in Table 8. The Centro-American

TABLE 8

Copulae observed in crosses between Centro-American (CA) females and Andean-South Brazilian (ASB) males strains. The third chromosome marked by Minute (CA), ebony (CA), and Delta (ASB) mutations
Control

	Number	Copulae with CA	Copulae with ASB	Number of hybrids tested	Chi-square	P
		Tests of Minute-ebony (Centro-American) females				
P	20	16	4	20	6.05	0.02–0.01
		Backcrosses to Centro-American parent—tests of hybrid females				
BC_3	20	16	4	23	6.05	0.02–0.01
		Backcrosses to Andean-South Brazilian parent				
BC_3	20	2	18	24	11.25	<0.01
		Tests of Delta (South Brazilian) males				
P	20	2	18	20	11.25	<0.01

Minute-ebony females prefer, as expected, Centro-American (Honduras) males, and the Delta South Brazilian males are preferred by South Brazilian (Angra) females. The "control" females from the progenies of the third backcross behaved like the females of pure subspecies.

Hybrids between the Amazonian and Andean-South Brazilian races: Only the effects of the X chromosomes in the progenies of the hybrids between these subspecies have been studied. Wild-type females of South Brazilian (Angra) subspecies were crossed to white-eyed males of the Amazonian (Belem) subspecies. As indicated above, the white gene behaves as a semidominant in this species. The F_1 hybrid females from this cross accept males of no subspecies (EHRMAN 1960b). (Backcross progenies were obtained by etherizing the females and immediately placing them with mature, unetherized males. The males will then approach and will often mount the females while they are still partly anesthetized. Subsequently, the females deposited fertilized eggs.) The F_1 hybrid males seem to be accepted significantly more frequently by females of the maternal subspecies (South Brazilian).

The behavior of the backcross progenies is interesting in that the preference for the subspecies of the recurrent parent grows only slowly or not at all. The females coming from the third backcross to the Amazonian parent still accepted Amazonian and South Brazilian males about equally. The same is true of the

backcross males. It appears that the South Brazilian X chromosome contains genetic modifiers that are strong enough to neutralize the Amazonian genes in the remainder of the genome.

DISCUSSION

The genetic architecture of reproductive isolating mechanisms is known only in very few instances. DOBZHANSKY (1936) studied the hybrid sterility and TAN (1946) studied the sexual isolation between the sibling species, *D. pseudoobscura* and *D. persimilis*. The sterility and the sexual isolation proved to be similarly constructed: they are due to summation of the effects of polygenes borne in all the chromosomes which those species have (except for the tiny fifth chromosome which was not studied). The subspecies, or incipient species, of *D. paulistorum* are also sexually isolated, and they also produce sterile male hybrids. But here the genetic mechanisms underlying the sterility and the sexual isolation are quite different. The evidence, as regards the cause of this sterility, is consistent with the view that any female which carries any mixture of the chromosomes of different subspecies deposits eggs giving rise to sterile male zygotes and to fertile female ones. The sterility of a male depends, in the backcrosses, upon the genetic constitution of his mother, and not on his own genetic constitution. In other words, the properties of an egg are determined, as far as the sterility of the backcross males is concerned, by the genotype present in it before meiosis, and not by that formed following fertilization. It should be noted that the presence in the mother of just one subspecies-foreign chromosome is already sufficient to make it produce sterile sons (EHRMAN 1960a).

The sexual isolation, which makes matings between females and males of the three subspecies of *D. paulistorum* much less likely to succeed than matings within a subspecies, is due to polygenes scattered in every one of the three pairs of chromosomes which these flies have. The subspecies of *D. paulistorum* resemble, in this respect, the pair of species, *D. pseudoobscura* and *D. persimilis*. As shown above, there exist, however, certain interesting differences between the subspecies.

Genes controlling the sexual preferences of both the male and female hybrids of the Centro-American and the Andean-South Brazilian subspecies, are concentrated particularly in the second chromosome (see Tables 5 and 6). As long as the Centro-American second chromosome is retained in the hybrids (its loss is prevented by selection for it each generation), repeated backcrosses to the Andean-South Brazilian subspecies do not neutralize the tendency to copulate with Centro-American individuals. On the other hand, as long as the Andean-South Brazilian second chromosome is retained in the hybrids (its loss by replacement is prevented by selection for it each generation), repeated backcrosses to the Centro-American subspecies do not dilute the tendency to copulate with Andean-South Brazilian flies. Note that none of the P values in Table 5 are significant.

The control experiments (Table 6) clearly validate the above conclusion in

that they indicate just the opposite situation. Both sexes show a pronounced preference for mating with individuals of the recurrent parental subspecies. The "BC_3s" to the Centro-American parent prefer Centro-American mates, and they lack the Andean-South Brazilian second chromosome. The "BC_3s" to the Andean-South Brazilian parent prefer Andean-South Brazilian mates, and they similarly lack the Centro-American second chromosome.

To express what is happening in the backcrosses in terms of percentage dilution of the alien chromosome, one might say that in repeated backcrosses between subspecies "A" and subspecies "B", where subspecies "B" is the recurrent parent, by the time the third backcross generation is reached, the percentage of "A" in the entire genome should be about 20.8 percent, while that of "B" is approximately 79.2 percent. In the controls, the theoretical percentage of "A" is 4.2 percent and that of "B" is 95.8 percent (see EHRMAN 1960a; Table 1 for details).

Some of the genes controlling the sexual preferences of the Centro-American and the Andean-South Brazilian subspecies and of their hybrids, are carried also in the third chromosome and in the X chromosome, as is shown by the data in Tables 7–9. The sexual preferences of the Centro-American vis-a-vis the Amazonian subspecies show, in a way, a more complex genetic structure (Tables 1–4). No one chromosome is here clearly more influential than the others. In the backcrosses, both sexes of the hybrids prefer and are preferred by the opposite sex of the subspecies which serves as the recurrent parent in the backcross. For the combination Amazonian × Andean-South Brazilian subspecies, only the effects of the X chromosome were studied, and even these not in a satisfactory manner, on account of the paucity of genetic markers (Table 9). The X chromosome clearly contains some genes determining the sexual preference, but most likely the autosomes would show such genes also.

A more detailed analysis of the genetics of the sexual isolation in *D. paulis-*

TABLE 9

Copulae observed in crosses between Andean-South Brazilian (ASB) females and Amazonian (AM) males strains. The first chromosome marked by a white (Am) mutation

	Number	Copulae with Am	Copulae with ASB	Number of hybrids tested	Chi-square	P
			Tests of hybrid females			
F_1	These females do not accept *any* males (EHRMAN 1960b)*					
			Backcrosses to Amazonian parent			
BC_1	20	5	15	62	4.05	0.05–0.02
BC_2	21	6	15	44	3.06	0.10–0.05
BC_3	20	13	7	55	1.25	0.30–0.20
			Tests of hybrid males			
F_1	20	4	16	66	6.05	0.02–0.01
			Backcrosses to Amazonian parent			
BC_1	20	12	8	62	0.45	0.70–0.50
BC_2	20	5	15	48	4.05	0.05–0.02
BC_3	20	8	12	43	0.45	0.70–0.50

* With the reciprocal cross, Amazonian ♀♀ × Andean-South Brazilian ♂♂, 18 out of 20 copulae observed were with Andean-South Brazilian ♂♂, and two were with Amazonian ♂♂ ($P<0.01$).

torum will have to await the availability of more genetic markers than are at our disposal at present. Although, as stated in the discussion of the experimental procedures, crossing over is probably largely suppressed in the subspecies hybrids, a better control over this point would clearly be desirable. The different genetic architecture of the hybrid sterility and of the sexual isolation is, in any case, beyond doubt. Which of these isolating mechanisms is more important is an interesting problem but only speculation is now possible. Sexual isolation is, adaptively, a more economical method than hybrid sterility, since it avoids the production of useless, sterile progeny. It is nevertheless likely that the peculiar chromosomal-cytoplasmic mechanism of sterility arose first, in the allopatric subspecies becoming adapted to different environments in their respective geographic areas. Sexual isolation would then be built by natural selection as a reinforcement of the bar to gene exchange between the subspecies. When this isolation became strong or complete, the subspecies, or incipient species, became able to coexist sympatrically, as they have been found to do in at least three different localities (DOBZHANSKY and SPASSKY 1959).

SUMMARY

The subspecies (or races, or incipient species) of *Drosophila paulistorum* show a pronounced sexual isolation. Matings between the subspecies succeed much less frequently than do those within a subspecies. The genetic basis of the sexual isolation has been studied in the hybrids between the Centro-American, Amazonian, and Andean-South Brazilian subspecies. Crosses have been made in which the distribution of a certain pair of chromosomes was followed with the aid of mutant genes which served as genetic markers. The sexual preferences were studied in the F_1 hybrids between the subspecies, and also in a series of backcrosses to each of the parental subspecies. A technique of direct observation was employed. The evidence thus obtained shows that the sexual isolation is fostered by factors distributed over all the chromosomes. The polygenes controlling the sexual preferences seem to produce additive effects, the sum of which makes the bar to crossing nearly complete between the subspecies. The sterility of the male hybrids between these subspecies has been previously shown to be due to a quite different genetic mechanism (EHRMAN 1960a). It is conjectured that the hybrid sterility is here the older of the two isolating mechanisms, but the sexual isolation is more effective in preventing gene exchange between the natural populations of the subspecies.

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COMPLEMENTATION STUDIES WITH ISOLEUCINE-VALINE MUTANTS OF NEUROSPORA CRASSA[1]

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IN recent years a number of investigators have presented the results of experiments concerned with complementation between mutants defective for a single genetic character. These results and their probable significance have been reviewed and discussed by BEADLE (1960), CASE and GILES (1960), FINCHAM (1960), and YANOFSKY and ST. LAWRENCE (1960).

The present work includes an intragenic complementation study at each of two loci concerned with isoleucine-valine biosynthesis. It was hoped that certain features of the complementation interaction could be clarified by a rather extensive program of testing. Specifically, understanding of the following problems was sought. Are the subgroups, of which the complementation map is composed, discrete? Does each genetic locus have associated with it a unique and characteristic pattern of such subgroups? Would linearity of the complementation map be observed in these systems?

MATERIALS AND METHODS

Induction and selection: Mutants were obtained by the "inositol-less death" selection procedure described by LESTER and GROSS (1959). The strain used in all induction experiments was *inos* 89601a which originated in a sample of nitrogen mustard treated spores descended from the standard wild cross Em 5256A × Em 5297a. Conidia were harvested from cultures grown at 35° on 20 ml of the complete medium described by HOROWITZ (1947).

The mutagens used, conditions of treatment and appropriate references are given in Table 1. The conidial concentrations at the time of treatment, as determined by optical density and comparison to a standard curve, were in the range $3–10 \times 10^7$ conidia per ml. All chemical treatments were terminated by dilution into cold M/15 phosphate buffer of *p*H 7.0. In all experiments, except 1, 2 and 3, the shaking step recommended by LESTER and GROSS (1959) was included.

All isoleucine-valine mutants were obtained on plates overlayed (after 3–4 days selection) with 5 ml of a minimal medium described by VOGEL (1956) to which was added agar 6.5 mg/ml, glucose 2.0 mg/ml, glycerol 2.0 mg/ml, sorbose

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TABLE 1

Mutagenic treatments

Mutagen	Abbrev.	Conc. or dose	Duration of treatment	Ml of conidial suspension treated	Measured conidial survival	References
Ultraviolet light*	UV	....	50 sec	10	30%	
X-irradiation†	X-ray	2500r/min	40 min	20	20%	
Nitrogen mustard (methyl-bis [beta-chloroethyl]-amine hydrochloride)	NM	0.0025 M	30 min	20	..	KÖLMARK and WESTERGAARD 1953
5-bromodeoxyuridine‡	5-BDU	....	..	..	..	
Tert-butyl-hydroperoxide	TBHP	0.09 M	30 min	20	..	DICKEY, CLELAND and LOTZ 1949
Ethyleneimine	ETIM	0.05 M	40 min	20	..	KÖLMARK and WESTERGAARD 1953
β-propiolactone	β-PL	0.004 M	30 min	20	..	SMITH and SRB 1951
Hydrogen peroxide with formaldehyde	HP+F	0.06 M H_2O_2 0.03 M HCHO	30 min	20	..	KÖLMARK and WESTERGAARD 1953
Nitrous acid§	HNO_2	0.005 M	96 min	11.6	..	KÖLMARK and DESERRES 1960

* The dose was delivered by a 30 Watt General Electric Germicidal Lamp to a suspension in an open petri dish (radius 4.5 cm) 9 cm from the lamp.
† The dose was delivered by a 250 kv Westinghouse Industrial X-ray Unit with no filtration to a suspension in an open petri dish 36 cm distant from the window.
‡ Conidia were harvested from cultures grown on 20 ml of HOROWITZ complete medium (HOROWITZ 1947) supplemented with 200μg/ml 5-BDU.
§ To initiate treatment four mg of $NaNO_2$ were added to the conidial suspension in 0.1 M acetate buffer (*p*H 4.6).

15 mg/ml, inositol 1 mg/ml, L-isoleucine 200 μg/ml and L-valine 200 μg/ml. Valine mutants were obtained from plates receiving an overlay similar to the above except that isoleucine and valine were replaced by 3.0 mg/ml casamino acids and the inositol concentration was 35 μg/ml.

Complementation tests: Complementation tests were regularly performed by a modification of the method of CATCHESIDE and OVERTON (1958). Large petri dishes (radius 7 cm) were used which contained 50 ml of Vogel's medium supplemented with sorbose 5.0 mg/ml, sucrose 1.0 mg/ml, inositol 10 μg/ml and agar 20 mg/ml. Conidial suspensions were prepared from cultures freshly grown on complete medium. Although conidial concentrations were not usually measured, complementation results were found to be regularly reproducible as long as the suspensions were visibly turbid (i.e., above 5×10^6 conidia/ml). Tests carried out with more dilute suspensions were found to be unreliable. Inocula were spotted on the plates with a wire loop, each spot receiving conidia from two mutants. As many as 45 spots could be placed on individual plates. These were stored at 25° and growth was recorded daily. Overgrowth of spots by neighboring, early-positive tests usually limited the usefulness of plates to from six to ten days.

Another, more sensitive, method was also employed for complementation testing. Small test tubes (75 mm by 10 mm), each containing 1 ml of Vogel's minimal medium supplemented with inositol 10 μg/ml, sucrose 10 mg/ml, glycerol 10 mg/ml, L-valine 100 μg/ml, L-isoleucine 100 μg/ml and agar 20 mg/ml, were prepared. To each tube inocula from two mutants were added. After three days growth at 25°, conidia were transferred to corresponding tubes containing media identical to the above except for the omission of isoleucine and valine. Observations of complementation could then be recorded during a 20 day period. However, because of the considerable extra work involved for each test, the use of this method was limited to checking the results obtained by the plate method.

Crosses: Crosses were made on Westergaard medium (WESTERGAARD and MITCHELL 1947) which contained L-isoleucine 100 μg/ml, L-valine 100 μg/ml, inositol 10 μg/ml, and several other amino acids. Recombination frequencies were obtained by plating out appropriate dilutions of random spores on both isoleucine-valine supplemented, and minimal media. After 30 minutes of heating at 60°, the plates were placed at 35° for about 24 hours. Counts were made with a low power binocular microscope when the colonies were still quite small. Also determined was the percent of spores not germinating on plates supplemented with isoleucine and valine.

RESULTS

Mutant yields: In all, 612 mutants, allocated to 23 series (Table 2), were obtained. All mutants were capable of growth on medium supplemented with isoleucine and valine, but not on minimal medium. Each mutant was designated by two numbers, the first indicating the series in which it was obtained and the second the order in which it was found in that series. Thus, mutant 18–13 was the 13th mutant found in series 18. The mutants of Experiment I were unique in that they were obtained from plates overlayed with minimal medium contain-

TABLE 2
Yields and distribution of mutants

Exp.	Series	Mutagen	Colonies picked	Number of mutants	Percent yield	Distribution				
						val-1	*val-2*	*iv-1*	*iv-2*	*iv-3*
I	1	UV	49	8	16.3	4	4	0	0	0
II	2	UV	561	20	3.6	0	0	0	1	19
III	3	UV	97	2	2.1	0	0	0	0	2
IV	4	UV	450	262	58.2	0	0	0	4	258
V	5	UV	53	23	43.4	0	0	0	1	22
VI	6	UV	101	56	55.5	0	0	0	0	56
VII	7	X-ray	18	1	5.6	0	0	0	1	0
	8	NM	246	3	1.2	0	0	0	1	2
	9	HP+F	400	0	0	0	0	0	0	0
	10	UV	400	15	3.8	0	0	1	4	10
	11	spontaneous	400	1	.25	0	0	0	0	1
	12	5-BDU	408	1	.25	0	0	0	1	0
VIII	13	UV	400	18	4.5	0	0	12	3	3
	14	X-ray	100	3	3.0	0	0	1	2	0
	15	NM	296	49	18.2	0	0	40	5	4
	16	TBHP	95	4	4.2	0	0	0	0	4
	17	ETIM	180	14	7.8	0	0	12	0	2
	18	β-PL	273	13	4.8	0	0	4	0	9
	19	HP+F	400	23	6.0	0	0	5	9	9
IX	20	UV	75	27	36.0	0	0	0	17	10
	21	NM	106	9	8.5	0	0	1	3	5
	22	HNO_2	30	2	6.7	0	0	1	0	1
	23	HP+F	465	58	12.5	0	0	1	42	15
Totals				612		4	4	78	94	432

ing casein hydrolysate, while in all other experiments an isoleucine-valine supplement was used.

Factors such as age of harvested conidia, conidial concentration at time of mutagen treatment, conidial concentration at time of plating and duration of selection are all expected to affect the yield of mutants (LESTER and GROSS 1959; KÖLMARK and WESTERGAARD 1953). Each of the experiments was known to vary with respect to these factors, and this, at least partially, accounts for the significant differences in distribution of mutant types from experiment to experiment (Tables 2 and 4). For each of the series within Experiments VII, VIII, and IX, these factors were held very nearly constant, from one series to the next, and only the mutagens used were changed.

Grouping of mutants by complementation: On the basis of complementation tests all isoleucine-valine mutants were placed in three groups, and the valine mutants into two additional groups. These have been designated[3] *iv-1*, *iv-2*, *iv-3*,

[3] The designation *iv-1* was first used by BARRATT, NEWMEYER, PERKINS and GARNJOBST (1954) as an assignment for mutant 16117 and its possible alleles. The designation *iv-2* was also introduced by these authors for alleles of mutant 39709, which on the basis of unpublished data was closely linked to 16117. The use of *iv-1* in the present work is consistent with this original usage. However 39709 was found to be heterokaryon incompatible with the mutants used in this study and there is no additional mention of it in the literature. Therefore it is not certain that present and original usage are consistent.

val-1 and *val-2*. All tests involving two mutants from different groups were always positive within a day after inoculation. The data from all intergroup tests are summarized in Table 3. The distribution of mutants, obtained in each series, among the various groups, is given in Table 2.

No complementation was observed between members of either the *val-1* or *val-2* group. Mutants in the *iv-1* group were all observed to grow on minimal medium between four and six days. For the other groups, the proportion of mutants displaying growth on minimal medium before five days was not more than a few percent of the total number. Among the 1,012 *iv-1* × *iv-1* pairwise complementation tests that were performed, several were positive 2–3 days before either mutant alone revealed growth. However, because of the complication of slow growth on minimal medium, this study was not pursued further.

The complementation behavior of isoleucine-valine mutant 16117, obtained by BEADLE and TATUM (1945), clearly placed it in the *iv-1* group. This mutant, in addition, displayed the slow growth on minimal medium characteristic of all *iv-1* mutants. It has been shown (ADELBERG and CAUGHLIN 1955) that 16117 accumulates the intermediates α,β-dihydroxyisovaleric acid and α,β-dihydroxy-β-methylvaleric acid (Figure 1), and has greatly diminished dehydrase activity with respect to these compounds (MEYERS and ADELBERG 1954). It is inferred from this evidence, and the recombination data to be presented, that *iv-1* mutants are defective in the dehydrase step of isoleucine-valine biosynthesis.

Complementation tests carried out on minimal medium supplemented with either L-valine alone, or L-isoleucine alone, gave results indistinguishable from tests carried out in the usual manner on unsupplemented minimal medium.

The iv-2 *group:* The 94 mutants allocated to the *iv-2* group were tested in 832 pairwise combinations. Eighteen of these mutants were found to complement with at least one other in the group. All possible pairwise tests among these 18 were performed. On the basis of the results a completely self-consistent linear complementation map was formulated (Figure 2). The mapping procedure is as follows: Results of pairwise growth tests are scored in the form of a matrix of positive and negative responses. Mutants are found which do not complement

TABLE 3

Intergroup complementation tests

Groups	Number of tests performed (all positive)
iv-1 + *iv-2*	620
iv-1 + *iv-3*	355
iv-1 + *val-1*	77
iv-1 + *val-2*	78
iv-2 + *iv-3*	1445
iv-2 + *val-1*	35
iv-2 + *val-2*	39
iv-3 + *val-1*	144
iv-3 + *val-2*	152
val-1 + *val-2*	16

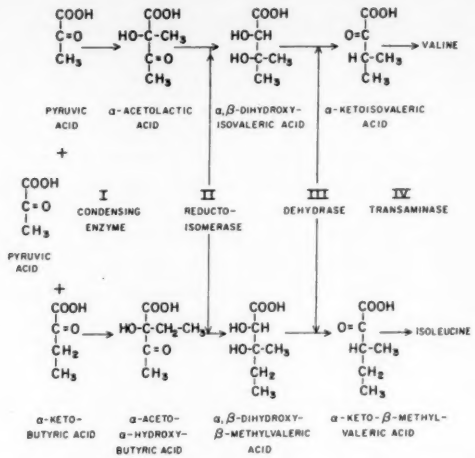

FIGURE 1.—Current concept of metabolic pathways for isoleucine and valine biosynthesis. (Based on WAGNER, RADHAKRISHNAN and SNELL 1958 and RADHAKRISHNAN, WAGNER and SNELL 1960).

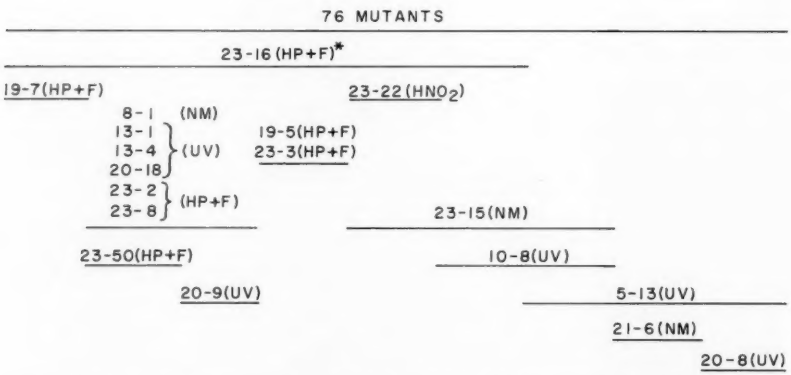

FIGURE 2.—Complementation map of the *iv-2* group. The individual mutants belonging to each subgroup, along with their mutagenic origins, are listed. The abbreviations used are: ultraviolet light, UV; nitrogen mustard, NM; hydrogen peroxide with formaldehyde, HP+F; and nitrous acid, HNO_2. The mutagenic origins of the "complete overlap" mutants are given in the text. *Mutant 23–16 complemented after three days with 5–13, but only after seven and six days with 21–6 and 20–8 respectively.

with two or more mutants which, in turn, do exhibit complementation. These noncomplementing or "overlap" mutants provide a basis for the construction of an ordered map. All mutants having the same pattern of complementation are placed in one subgroup represented on the map by a line. Noncomplementing

subgroups are represented by overlapping lines, whereas lines representing complementing subgroups are not overlapped. If the data allows every subgroup to be designated by a single uninterrupted line, the complementation map is said to be linear. However, the positions of all the subgroups were not uniquely determined, and therefore more than one linear map could have been derived from the data. For instance, the positions of 21–6 and 20–8 could have been reversed. Almost all positive responses were initiated between three and five days after inoculation. There was some tendency for heterokaryotic growth of a mutant combination to be more vigorous as the apparent number of subgroups separating mutants on the map increased. However, one case of exceptional behavior was clearly established in repeated tests. Mutant 23–16 initiated a positive response with 5–13 three days after inoculation, whereas with the more "distant" 21–6 and 20–8, complementation was not observed until seven and six days, respectively.

The 76 completely overlapping mutants had the following mutagenic origins: 42, hydrogen peroxide with formaldehyde; 23, ultraviolet light; seven, nitrogen mustard; three, X-rays; and one, bromodeoxyuridine. Of particular interest were six mutants, representing three different mutagenic origins, which displayed identical complementation patterns (Figure 2 second subgroup from left). This result was characteristic, to a greater extent, of mutants in the *iv-3* group, and therefore its possible significance will be discussed in the following sections.

The iv-3 *group*: On the basis of a program of testing in which the 432 *iv-3* mutants were subjected to about 10,500 pairwise complementation tests, the map shown in Figure 3, was formulated. The complementation behavior of 428 of the mutants was completely consistent with this linear representation, but the remaining four could not be reconciled with it. Three of these would not conform to any linear representation. The properties of these mutants are discussed below.

The most interesting feature of the results was that the complementation map contained only a few subgroups, i.e. among 428 mutants there existed only six patterns of complementation. However, 325 of these were C mutants derived from UV treatment. This representation with only six subgroups was initially

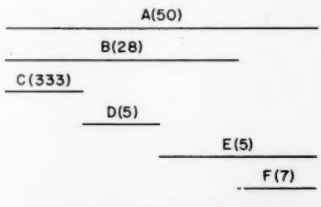

FIGURE 3.—Complementation map of the *iv-3* group. Each subgroup is given a letter designation after which is the number of mutants found in that subgroup. *Four *iv-3* mutants displayed patterns of complementation incompatible with this representation. Three of these could not be reconciled with any map in which all the subgroups are represented by uninterrupted line segments. For further details, see text.

observed early in the testing program when only UV induced mutants were available. Of the 52 non-UV mutants subsequently obtained (Table 4) 25 did not complement and were placed in subgroup A, 23 could be placed in one of the other five established subgroups, and four gave exceptional results.

The number of possible pairwise tests (T), where n is the number of mutants tested, is $\frac{1}{2}\, n(n-1)$. When n is 432, T is 93,096. The number of tests actually performed, 10,500, was only 11 percent of this total. Therefore, it would seem advisable to mention some of the factors involved in choosing those tests which were carried out. Initially mutants 4–1 to 4–65 were tested in all pairwise combinations, and then arranged in classes according to their pattern of complementation. Mutants representing each class were next used as testers to classify systematically the other mutants. Any newly tested mutant not conforming to the established pattern of complementation, was, after rechecking, itself used as a tester. Classifications thus arrived at were subjected to numerous cross-checks. Although only 3,000 of the 55,278 possible C + C tests were actually performed (all giving negative results), it seemed unnecessary to prolong this phase of the testing program. The omitted C + C tests account for more than half the total tests not carried out.

TABLE 4

Distribution of iv-3 *mutants found in successive selection experiments**

Series no.	Mutagen	A	B	C	D	E	F	Exceptions	Totals
1	UV	0	0	0	0	0	0	0	0
2	UV	3	5	1	5	0	5	0	19
3	UV	0	1	1	0	0	0	0	2
4	UV	7	4	246	0	1	0	0	258
5	UV	1	1	20	0	0	0	0	22
6	UV	2	2	50	0	2	0	0	56
7	X-ray	0	0	0	0	0	0	0	0
8	NM	1	1	0	0	0	0	0	2
9	HP+F	0	0	0	0	0	0	0	0
10	UV	2	3	4	0	0	1	0	10
11	Spont	0	1	0	0	0	0	0	1
12	5-BDU	0	0	0	0	0	0	0	0
13	UV	3	0	0	0	0	0	0	3
14	X-ray	0	0	0	0	0	0	0	0
15	NM	1	2	0	0	0	0	1	4
16	TBHP	3	0	1	0	0	0	0	4
17	ETIM	0	1	0	0	0	1	0	2
18	β-PL	6	0	1	0	0	0	2	9
19	HP+F	3	2	3	0	1	0	0	9
20	UV	7	0	3	0	0	0	0	10
21	NM	2	1	2	0	0	0	0	5
22	HNO_2	0	0	1	0	0	0	0	1
23	HP+F	9	4	0	0	0	1	1	15
	Total	50	28	333	5	4	8	4	432
	UV	25	16	325	5	3	6	0	
	non-UV	25	12	8	0	1	2	4	

* Several mutants, which grew on minimal medium to the extent that classification was interfered with, were not included.

There tended to be a correlation between the vigor of heterokaryotic growth and the pair of subgroups represented in the heterokaryon. Thus the complementation interactions between mutants in the F and C groups were characterized by abundant growth starting on the first day after inoculation. The same was true of E + C and F + D, whereas E + D heterokaryons took about two days to initiate growth. B + F positive tests were variable in time of initiation but occurred, on the average, about five days after inoculation. Growth in these cases was always scanty. The tube method for detecting complementation (see Materials and Methods) was used mainly for checking the B + F combinations, and also D + C tests which grew after an average of 11 days, with even more variability, and gave exceedingly scanty growth response.

Two of the exceptional mutants, 8–10 and 23–56, did not complement with each other and were in all respects very similar. Neither complemented with A, E or F mutants. Both complemented readily with all B mutants tested. 18–10 gave a positive response with 24 B mutants after an average of about two days, whereas 23–56, in tests with 22 B mutants took about four days. Both complemented slowly with 60 percent of the C mutants (an average of five days in each case) and would not complement at all with 40 percent of the C's. To represent these mutants on the complementation map would inevitably require two nonadjacent line segments as well as a subdivision of group C. The new subgroups arising because of the exceptional mutants certainly have an equal claim to letter designations. However, in order to make these assignments with any surety, it would have first been necessary to have established the complete complementation spectrum of all the exceptional mutants. To accomplish this, with the required resolution, using the techniques now available, would have been a considerable undertaking.

The two remaining exceptional mutants (15–7 and 18–13) did complement with each other. 15–7 acted, in general, like a C mutant, although unlike C's, it complemented with about 40 percent of the B mutants after an average of eight days. Although there was insufficient data to completely characterize mutant 18–13, its behavior clearly did not fit the established pattern and was in addition incompatible with linearity.

Mutants representing the *iv-1*, *iv-2* and *iv-3* groups, in addition to *inos* 89601, have been assayed for the enzymatic ability to convert pyruvic acid to α-acetolactic acid essentially as described by RADHAKRISHNAN and SNELL (1960). A representative mutant in both the *iv-1* and *iv-2* groups, as well as *inos* 89601, displayed substantial activity for these reactions, whereas *iv-3* mutants had little if any activity. This preliminary evidence suggests that *iv-3* mutants are blocked in Step I (Figure 1) of the biosynthetic pathway.

An *iv* mutant, 7110, has been shown by WILSON and ADELBERG (1957) to accumulate several organic acids, the principal ones being citramalic and α,β-dimethylmalic acids. By complementation this mutant belonged to the *iv-3* group. The explanation for these accumulation products is unknown. Further work on the enzymology of Step I is now in progress.

Crosses: Preliminary crosses were performed in order to check to some extent

the groupings indicated by complementation tests. Mutant 16117A (*iv-1*), assigned to the right arm of linkage group V (BARRATT *et al.* 1954), was crossed to members of each of the five groups and proved to be unlinked or distantly linked to mutants and the *val-1*, *val-2* and *iv-3* groups. In the 12 fertile crosses between 16117 and various *iv-2* mutants, linkage was observed in every case. The values varied between 2.6 and 8.6 map units and had an average of 6.3. Spore germination in these crosses was between 40 and 90 percent. In the single fertile cross to another *iv-1* mutant (10–9), no prototrophs were observed among approximately 2,000 germinated random spores. In this case there was 21 percent germination. Therefore mutants 16117 and 10–9 represent defects at either identical or very closely linked sites. This evidence suggests that the three *iv* groups represent three genetic loci. Two of these (*iv-1* and *iv-2*) are in linkage group V, about 6.3 map units apart, and both are unlinked to *iv-3*.

DISCUSSION

The most apparent conclusion from these interallelic complementation studies is that all of the *iv-2* data and the large majority of the *iv-3* data are compatible with simple linear complementation maps. In addition, the data are in general accord with WOODWARD's observation (WOODWARD 1959) that the vigor of heterokaryotic growth between two mutants increases with "distance apart" on the complementation map. At the present stage of understanding it is difficult to know how to assess the importance of the four *iv-3* mutants whose complementation behavior differs from the established pattern, and especially the three of these which will not conform to a linear map.

On the basis of these results, the hypothesis may be formulated that *each locus has associated with it a unique and characteristic pattern of discrete complementation subgroups*, and that the observations in a complementation study reflect this pattern. It is, furthermore, assumed that this pattern reflects the molecular structure of the enzyme controlled by the locus.

The main support for this hypothesis is the result that the very large number of *iv-3* mutants which were found, proved after extensive testing, to belong to a relatively small number of subgroups. Also of consequence was the finding that, of 18 complementing *iv-2* mutants, six fell into one subgroup. It is predicted that the number of *iv-2* subgroups will continue to remain small with the accumulation of more *iv-2* mutants.

The validity of the hypothesis depends, in part, on how safely grounded are the subgroup assignments. It can be asked, with what degree of certainty are the mutants, allocated to a single subgroup, identical to each other in complementation behavior? When successive mutants within a subgroup are tested against mutants in subgroups that are not adjacent on the map, very uniform positive results are obtained. However, when the tests involving "adjacent" subgroups are considered, qualifications do arise. These tests give the least vigorous heterokaryotic growth and are, in fact, often variable in expression. However, increased care and repetition tends to minimize variations. It therefore seems probable that

it is the insensitivity of the procedures generally used for detecting complementation, when applied to weakly positive tests, rather than a lack of subgroup discreteness that accounts for this variability.

A note of caution should be included concerning limitations of the heterokaryotic growth assay as a measure of the primary complementation interaction which undoubtedly occurs at or close to the molecular level. The possibility must be entertained that, if a more direct feature of complementation such as enzymatic activity, were regularly screened for, the observed pattern of interactions at a given locus would be different. Certainly with the greater resolution offered by enzyme assay the observed pattern could be modified.

Also it is easy to envision situations in which enzymes active *in vitro* would not be active *in vivo* and thus not support growth. For example experiments by SUSKIND and KUREK (1959) indicate that a potentially active form of tryptophan synthetase is inhibited by high levels of metal ion in the cytoplasm. If enzymes of this type were to form by complementation, the growth and enzymatic assays would yield conflicting results.

Another limitation of the growth assay arises because mutants which are capable of some growth on minimal medium are difficult to handle experimentally. The selective omission of this class of mutants from testing schedules may lead to a bias in the observed complementation patterns. In the present experiments a few mutants were left out of the tabulations because their slow growth on minimal medium interfered with the tests necessary for classification. However, from the tests that could be performed, there was no indication of unusual complementation behavior.

Ideally, a completely general test for complementation would be independent of large increases in enzymatic activity and measure interaction, *per se*. Present methodology precludes a consideration of the generality of interaction between products of allelic mutants coexisting in a heterokaryon. Although, the application of immunological or physiochemical tests seems impractical at this time, the study of enzymes produced in heterokaryons of wild type and mutant may give some indication of the generality of interaction.

Interpretations of the results are subject to further reservations imposed by a lack of knowledge concerning two factors which could certainly bias the distribution of mutant types. These are, mutational "hot spots" (BENZER and FREESE 1958) and selection (during the experiments by which the mutants were obtained). In phage T_4 it has been shown that the spectra of "hot spots" at a genetic locus, induced by unrelated mutagens, show little, if any, overlap (BRENNER, BENZER and BARNETT 1958). Therefore, the observation that 23 of 27 non-UV induced *iv-3* mutants gave the same few complementation patterns observed for UV induced mutants, argues against the possibility that similarity of behavior among mutants primarily reflects "hot spot" contributions.

No estimation can be made at present concerning the effect of selection on the distribution of mutants among the various subgroups.

WAGNER, SOMERS and BERGQUIST (1960) have recently reported the results of experiments also concerned with isoleucine-valine mutants of Neurospora.

They concluded that clustered in a single complementation group were three kinds of mutants. Those allocated to one end of the complementation map were deemed defective in the reductoisomerase step (Figure 1), and those at the other end, in the dehydrase step. Mutants located in the middle were postulated to be defective in both steps. Their recombination data were used to assign these mutants to a small region of the genetic map (about four map units) in the right arm of linkage group V. One mutant, 16117, also used in the present study, was placed at the dehydrase end of their complementation map.

Our results place 16117 in what we have designated complementation group *iv-1*. All pairwise tests of these mutants with those we have placed in group *iv-2* were clearly positive within one day, without any indication of ambiguity (Table 3). Recombination data, albeit limited, indicate that mutants in group *iv-2* are similar to those reported by WAGNER, *et al.* (1960) which lack only the reductoisomerase and which cluster at the end distal from 16117 in their complementation map.

Thus it appears that the present results differ from those of WAGNER, *et al.* (1960) in that no mutants were found which exhibited complementation behavior corresponding to that of mutants placed in the middle group described above. Whether this difference is attributable to differences in mutant selection or complementation testing techniques remains to be examined.

SUMMARY

A total of 616 mutants capable of growth on a medium supplemented with isoleucine and valine, but not on minimal medium, were obtained through the use of nine different mutagens. On the basis of heterokaryon complementation tests all of the mutants could be allocated to five groups. These groups were designated *val-1*, *val-2*, *iv-1*, *iv-2* and *iv-3*. Mutants in the *val-1* and *val-2* groups required valine as the sole supplement. Members of the *iv-1* group were probably blocked in the dehydrase step of isoleucine-valine biosynthesis, and were characterized by slow growth between four and six days after inoculation on minimal medium. An extensive program of complementation testing was performed among mutants within both the *iv-2* and *iv-3* groups. The results of these tests allowed, in each case, the formulation of a complementation map. However, three *iv-3* mutants were found which were not compatible with a linear pattern. An interesting feature of the *iv-3* studies, and to some extent of the *iv-2* studies, was the finding that among successive mutants the same patterns of complementation interaction often recurred. Mutants with the same complementation behavior constitute a complementation subgroup. Each of these subgroups usually contained mutants representative of several different mutagenic treatments. The hypothesis was considered that this observed clustering reflects an intrinsic property of the genetic locus—namely that each locus has associated with it a unique pattern of discrete complementation subgroups.

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CARBON DIOXIDE SENSITIVITY IN DROSOPHILA AFFINIS AND DROSOPHILA ATHABASCA[1]

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SENSITIVITY to carbon dioxide in *Drosophila melanogaster*, discovered in 1937 by L'HÉRITIER and TEISSIER, has been the object of considerable research by L'HÉRITIER and his co-workers in France (L'HÉRITIER 1948, 1951, 1958). Since no study had previously been made in species other than *D. melanogaster*, it was decided to investigate the occurrence of CO_2 sensitivity in several of the American Drosophila species. All testing with CO_2 has been conducted at the *D. melanogaster* standard conditions, *viz.*, 15 minutes at 13°C with pure CO_2 (PLUS 1954). Following the treatment, any fly which cannot right itself and walk after 15 minutes in an atmosphere of free air is classified as sensitive.

During the last few years CO_2 sensitivity has been encountered in laboratory stocks and/or wild-caught flies of several native American species: *Drosophila affinis* from Georgia, Minnesota, Missouri, Nebraska, and Tennessee; *D. algonquin* from Minnesota and Nebraska; *D. athabasca* from Alaska and Minnesota; *D. pseudoobscura* from Nebraska; *D. robusta* from Nebraska; *D. tolteca* from Nicaragua and Mexico. In these species, CO_2 sensitivity has been found only rarely in *D. algonquin*, *D. pseudoobscura*, *D. robusta*, and *D. tolteca*. However, in *D. athabasca* from three to 20 percent of the wild-caught flies have been found to be CO_2 sensitive (Table 1), while in *D. affinis* the frequency of CO_2 sensitivity has been found to be somewhat greater—from 16 to 39 percent (Table 2). Entirely sensitive strains have been established in *D. affinis* and *D. athabasca*, and the nature of the inheritance of CO_2 sensitivity in these two species has been studied.

In addition, *D. melanogaster* collected in Nebraska has been found to be 1.6 percent CO_2 sensitive out of 6,300 flies tested. Also, MR. DOUGLAS LUND of the University of Nebraska (personal communication) has found CO_2 sensitivity in laboratory stocks of *D. azteca* from Mexico; *D. macrospina* from Arizona, Georgia, Mexico, Nebraska, New Mexico, South Carolina, and Texas; *D. pallidipennis* from Colombia; *D. prosaltans* from Mexico.

Studies of the genetic basis: L'HÉRITIER (1948) describes the mode of inheritance of CO_2 sensitivity in *D. melanogaster* as being maternal, though not strictly so, since males also transmit sensitivity, but with less efficiency. For many years CO_2 sensitivity was held as a clear case of cytoplasmic heredity and the word genoid was used in reference to the CO_2 sensitivity agent. Following the discovery

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TABLE 1

Incidence of CO_2 sensitivity in wild-caught Drosophila athabasca

Place	Time	Number tested	Sensitives	
			Number	Percent
Big Lake, Alaska	Aug., 1957	591	52	9
College, Alaska	Aug., 1957	441	38	9
Matanuska Valley, Alaska	July-Aug., 1957	47	2	4
Quetico-Superior Wilderness Research Center, Minnesota	July, 1959	677	134	20
Northfield, Minnesota	June, 1959	379	13	3
Hallock, Minnesota	July, 1959	35	5	14

TABLE 2

Incidence of CO_2 sensitivity in wild-caught Drosophila affinis

Place	Time	Number tested	Sensitives	
			Number	Percent
Georgia				
Alto	May, 1959	46	17	37
Chenocetah Mt.	May, 1959	61	15	24
Clayton	May, 1959	30	6	20
Lake Russell	May, 1959	49	16	32
Minnesota				
Northfield	June, 1959	79	19	24
Quetico-Superior Wilderness Research Center	July, 1959	21	7	33
Missouri				
Webster Groves	Sept., 1958	41	13	31
Nebraska				
Allen	June, 1959	61	24	39
Chadron State Park	June, 1957	163	41	25
Halsey	June, 1958	41	13	31
Humboldt	June, 1958	427	114	27
	Sept., 1958	266	55	21
	July, 1959	146	27	18
Lincoln	June-Aug., 1957	79	19	24
	June, 1958	128	32	25
	June, 1959	168	32	19
North Carolina				
Raleigh	May, 1959	80	21	26
South Carolina				
Greenville	May, 1959	53	18	34
Tennessee				
Sieverville	May, 1959	88	19	22
Gatlinburg	May, 1959	38	10	26
Smoky Mt. Park	May, 1959	140	32	23

of its infectious capacities (L'HÉRITIER and HUGON DE SCOEUX 1947) the term genoid seemed unjustified. The CO_2 sensitivity agent seems to fit every fundamental characteristic of a virus and, accordingly, since 1954, the word genoid has been replaced, and the CO_2 sensitivity agent has been called virus *sigma* (σ).

BRUN and SIGOT (1955) have described two types of CO_2 sensitive flies, stabilized and nonstabilized. The stabilized type corresponds to flies of pure sensitive stocks and is characterized by females which give rise to 100 percent sensitive offspring; males which produce a varying percentage of sensitive offspring, depending upon the genotype of the fly and the genotype of the virus; and a low yield of infectious virus. The nonstabilized type corresponds to flies which have either received their sensitivity from their father or artificially by inoculation. Nonstabilized flies are characterized by a high yield of infectious virus, by females that produce both sensitive and resistant offspring, and by males that do not transmit CO_2 sensitivity to their offspring. For a more complete discussion of the stabilized conditions, the reader is referred to L'HÉRITIER (1958).

Since CO_2 sensitivity was discovered in *D. athabasca* before being found in *D. affinis*, the studies of sensitivity in *D. athabasca* will be dealt with first in this paper.

Inheritance of CO_2 sensitivity in Drosophila athabasca: The strain of sensitive *D. athabasca* employed in this study originated from a single wild-caught female from the Matanuska Valley, Alaska, collected by DR. D. D. MILLER of the University of Nebraska, and designated as MV-4. The resistant strain, designated J-LP, was one derived from a cross between females from a Jackson Hole Biological Research Station, Wyoming, strain and males of a strain from Laurentides Park, Quebec.

L'HÉRITIER and TEISSIER (1938) in their attempts to determine the role of chromosomes in the hereditary transmission of CO_2 sensitivity in *D. melanogaster* employed mutant genes as markers for each of the autosomes. Because of the lack of marker genes in *D. athabasca*, a series of crosses was made to replace as much as possible the chromosomes of the sensitive strain with those of the resistant strain, the cytoplasm being supplied by the sensitive strain. The results of these crosses are given in Table 3.

Progeny from the cross of sensitive females by resistant males were 99 percent sensitive. Backcrosses of sensitive females to males from the resistant strain produced 100 percent sensitive offspring through the fourth backcross. The fifth backcross, for no evident reason, yielded no offspring, thus the series of backcrosses came to an end. In this series of backcrosses, the average percent of chromosomal material from the sensitive strain, MV-4, is expected, in the absence of differential survival and reproduction of combinations of the genes of the two strains, to decrease by one half at each generation. The progeny from the fourth backcross would be expected to possess only 3.125 percent chromosomal material from the sensitive strain, with the cytoplasm being supplied almost wholly by the sensitive strain. Nevertheless, sensitivity still remained at 100 percent.

The reciprocal cross, resistant J-LP females by sensitive MV-4 males, gave 27 percent sensitive offspring in a preliminary cross and 37.5 percent when the cross was repeated later. From the offspring of the second cross, 33 F_1 pair matings were attempted to determine whether flies inheriting sensitivity from their fathers could transmit this sensitivity to their offspring. Only 17 of the pairs remained

TABLE 3

Reciprocal crosses between sensitive and resistant Drosophila athabasca

Parents	Offspring Sens.	Offspring Res.	Percent sensitive	Expected percent chromosomal material from MV-4 in offspring
Original cross: MV-4♀ (sensitive) × J-LP♂ (resistant)				
MV-4♀ × J-LP♂	241	3	99	50.00
F_1 ♀ × J-LP♂	184	0	100	25.00
BC_1 ♀ × J-LP♂	219	0	100	12.50
BC_2 ♀ × J-LP♂	209	0	100	6.25
BC_3 ♀ × J-LP♂	50	0	100	3.125

Parents	Offspring Sensitive	Offspring Resistant	Percent sensitive
Reciprocal cross: J-LP♀ (resistant) × MV-4♂ (sensitive)			
1. J-LP♀ × MV-4♂	37	100	27.0
2. J-LP♀ × MV-4♂	98	261	37.5
$F_1 \times F_1$			
Both sensitive (one pair)	11	30	27.0
♀ Sensitive,			
♂ Resistant (3 pairs)	15	12	56.0
	34	84	29.0
	24	26	48.0
♀ Resistant,			
♂ Sensitive (2 pairs)	0	22	. . .
	13	50	21.0

alive at the time of CO_2 testing, and of these, only seven were ones in which one or both flies were sensitive.

There were two cases in which both male and female were sensitive; one of these yielded no offspring, but the other produced flies which were 27 percent sensitive. In three cases the female was sensitive and the male resistant. In the progenies of these there was a variable mixture of sensitive and resistant flies, the frequency of sensitivity ranging from 29 percent to 56 percent. The most interesting results were obtained from one of the two pair matings in which the female was resistant and the male sensitive. Although one of these pairs gave rise to only resistant offspring, the other pair mating produced flies which were 21 percent CO_2 sensitive. This latter result is in contrast to what is expected in similar crosses with *D. melanogaster*, in which L'Héritier and Teissier (1938) report that sensitivity is *not* transmitted by a male that inherited its sensitivity only from its father.

Inheritance of CO_2 sensitivity in Drosophila affinis: The sensitive strain of *D. affinis* employed in this study was derived from a CO_2 sensitive female collected at Humboldt, Nebraska, and has been designated as H-16. The resistant strain was derived from a female collected at Chadron State Park, Nebraska, and has been designated as CSP.

In *D. affinis*, as in *D. athabasca*, there were no marker genes available, and it was necessary to use a backcross procedure for replacing the chromosomes of the sensitive strain by those of the resistant strain, as outlined above for *D. athabasca*.

Sensitive H-16 females mated with resistant CSP males produced 100 percent CO_2 sensitive progeny (see Table 4). The series of backcrosses was carried through the sixth backcross, with 100 percent CO_2 sensitivity in each backcross progeny. In the progeny of the sixth backcross the average amount of chromosomal material from the sensitive strain is expected to be 0.78 percent. The results indicate that CO_2 sensitivity in *D. affinis*, as in *D. athabasca* and *D. melanogaster*, is inherited independently of the chromosomes.

In the reciprocal cross, resistant CSP females by sensitive H-16 males, the progeny were 98.6 percent CO_2 sensitive. Pair matings of resistant CSP females by sensitive F_1 males that inherited their sensitivity only from their father, produced some interesting, but not well understood, results. Six pairs of flies, of varying ages, were placed in vials of Synthetic S-101 medium (modified from PEARL, ALLEN and PENNIMAN 1926). After 7–10 days these parents were transferred to fresh vials of S-101 medium where they remained for seven days before being removed and tested with CO_2. Table 4 shows that of the six pair matings of the type described, no sensitive offspring appeared in the first vial, but varying percentages (8 percent to 21 percent) of sensitivity were found in the second vial. That sensitivity appeared at all in these offspring is contrary to what would be obtained in similar crosses with *D. melanogaster* (as was the case with *D. athabasca*). An attempt to observe these phenomena in repeated crosses of the above kind has given only negative or equivocal results. An additional seven F_1 sensitive males were mated with resistant CSP females. The males were about ten days

TABLE 4

Reciprocal crosses between sensitive and resistant Drosophila affinis

Original cross: H-16♀ (sensitive) × CSP♂ (resistant)				
Parents	Offspring Sens.	Res.	Percent sensitive	Expected percent chromosomal material from H-16 in progeny
H-16♀ × CSP♂	171	0	100	50.00
F_1♀ × CSP♂	178	0	100	25.00
BC_1♀ × CSP♂	210	0	100	12.50
BC_2♀ × CSP♂	14	0	100	6.25
BC_3♀ × CSP♂	119	0	100	3.125
BC_4♀ × CSP♂	18	0	100	1.56
BC_5♀ × CSP♂	39	0	100	0.78

Reciprocal cross: CSP♀ (resistant) × H-16♂ (sensitive)						
	Offspring					
	Vial no. 1		Vial no. 2		Percent sensitive	
Parents	Sens.	Res.	Sens.	Res.	Vial no. 1	Vial no. 2
CSP♀ × H-16♂	349	5	.	..	98.6	...
CSP♀ × F_1♂						
(6 pairs)	0	15	2	23	0.0	8.0
	0	56	3	34	0.0	8.1
	0	15	4	15	0.0	21.0
	0	12	6	33	0.0	15.3
	0	18	1	29	0.0	3.3
	0	19	4	22	0.0	15.3

old as adults when the matings were made and the flies were allowed to remain in the first vial for six days before being transferred to a second vial, where they were kept for four days before they were removed and tested with CO_2. However, of 550 offspring from these matings, none was sensitive.

Injection experiments with Drosophila affinis *and* Drosophila athabasca: To determine whether the CO_2 sensitivity agent in *D. affinis* and *D. athabasca* could be transmitted to resistant flies through inoculation, flies from resistant strains of these two species were injected with extracts of sensitive flies of the respective species. The injection procedures employed were those already described for work on CO_2 sensitivity in *D. melanogaster* (PLUS 1950, 1954).

In resistant *D. athabasca* (a mixed strain derived from the Jackson Hole Biological Research Station, Wyoming, and Iron River, Wisconsin, strains) injected with an extract prepared from sensitive flies from the MV-4 stabilized strain, sensitivity appeared in seven out of 25 injected flies 17 days after injection. The injected sensitive flies did transmit this sensitivity to ten out of 36 offspring. Unfortunately, since the offspring did not reproduce, it was not possible to establish a sensitive strain of *D. athabasca* by the injection procedure.

A resistant strain of *D. affinis* from Hopkinsville, Kentucky, was injected with an extract prepared from F_1 sensitive flies from a mating of resistant CSP females by sensitive H-16 male. Out of 42 injected flies five survived the injection procedure and all five became sensitive. Sensitivity appeared 29 days after injection. The injected females produced only four offspring, but among these there was one sensitive female with which a neosensitive strain of *D. affinis* has been established.

DISCUSSION

Certain similarities have been found in the CO_2 sensitivity phenomena in *D. melanogaster* on the one hand and *D. affinis* and *D. athabasca* on the other. It has been shown that in all three species CO_2 sensitivity tends to be maternally inherited, though not strictly maternal, since males can also transmit sensitivity. Moreover, by following a breeding procedure designed to replace the chromosomes of a sensitive strain with those of a resistant strain it has been shown in *D. affinis* and *D. athabasca* that, as in *D. melanogaster*, CO_2 sensitivity is inherited independently of the chromosomes. In addition, an extract derived from sensitive *D. affinis*, *D. athabasca*, and *D. melanogaster*, and injected into resistant strains of the respective species, with the subsequent development of CO_2 sensitivity symptoms in the injected flies, has demonstrated the infective nature of the prepared extract.

The most striking difference between the inheritance of CO_2 sensitivity in *D. melanogaster* on the one hand and *D. affinis* and *D. athabasca* on the other was the hereditary transmission in the latter two species of CO_2 sensitivity by males which have inherited it only from their fathers. In *D. melanogaster* such males, called nonstabilized males, never transmit sensitivity; whereas stabilized males, which have received their sensitivity from their stabilized mothers, are able to transmit the virus to some of their offspring, which are always of the non-

stabilized type. The stabilized condition is inherited only through the maternal line (L'HÉRITIER 1958).

Although attempts to repeat the transmission of CO_2 sensitivity by nonstabilized *D. affinis* and *athabasca* males have not produced meaningful results, *a priori* there is no reason why such transmission could not occur in these species. Such transmission by nonstabilized *D. affinis* and *athabasca* males indicates that there is a different relationship established between the virus and the fly from that which has been described for *D. melanogaster*. The nature of this different relationship is not yet known.

SUMMARY

Certain laboratory strains of *Drosophila affinis*, *D. athabasca*, and *D. tolteca* were found to contain CO_2 sensitive flies. CO_2 testing of wild-caught flies revealed CO_2 sensitivity in the following North American species of Drosophila: *D. affinis* from Georgia, Minnesota, Missouri, Nebraska, and Tennessee; *D. algonquin* from Alaska and Minnesota; *D. pseudoobscura* from Nebraska; and *D. robusta* from Nebraska.

Pure CO_2 sensitive strains of *D. affinis* and *D. athabasca* were established. Breeding experiments showed that CO_2 sensitivity in these species tends to be maternally inherited, and also that it is inherited independently of the chromosomes, as is true in *D. melanogaster*. A special feature of *D. affinis* and *D. athabasca*, in contrast to *D. melanogaster*, is the hereditary transmission of CO_2 sensitivity by males which have inherited their sensitivity only from their father.

An extract prepared from sensitive flies and injected into resistant flies demonstrated that the agent responsible for sensitivity in *D. affinis* and *D. athabasca*, as in *D. melanogaster*, can induce the development of sensitivity symptoms in the injected flies. However, a neosensitive strain was successfully established only in *D. affinis*.

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EXPRESSION AND STABILITY OF THE MARBLED ALLELE IN MAIZE[1]

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THE pigment-producing action of the R^r allele (colored aleurone, red seedling) in maize regularly is reduced among the offspring of heterozygotes carrying an allele, R^{st}, conditioning stippled aleurone and green seedling (BRINK 1956). The modified forms of R^r which arise through such intrachromosomal changes at or near the R locus are heritable. Genetic change of this kind has been termed paramutation (BRINK 1958a). The phenomenon has been demonstrated also in R^r heterozygotes in which another allele, R^{mb} (marbled aleurone, green seedling) was substituted for stippled (BRINK and WEYERS 1957).

The stippled and marbled factors are of considerable interest as sources of chromosomal determinants involved in these directed genetic changes at the R locus. An account of the stippled gene has been presented by ASHMAN (1960). The present study is concerned with the expression and stability of the marbled allele.

MATERIALS AND METHODS

The marbled gene employed in these investigations was introduced from a stock originally obtained from R. A. EMERSON into a highly uniform inbred line known as W22. After outcrossing the original R^{mb} strain to W22, the marbled segregates were backcrossed four or more times to the W22 inbred line, prior to their isolation in homozygous form by selfing.

When homozygous, the R^{mb} allele frequently causes formation in the aleurone of large, irregularly shaped, solidly colored spots on a colorless background. The degree of spotting is proportional to dosage. The scutellum is colorless. Marbled does not produce appreciable amounts of pigment in the vegetative tissues. Seedlings show traces of anthocyanin in the coleoptiles, but the roots remain colorless when exposed to light. The anthers and glumes on adult $R^{mb}R^{mb}$ plants are green.

The otherwise uniform marbled W22 stocks here employed were observed to vary strikingly in amount of aleurone spotting. To determine whether the observed variability was genetic, dark, intermediate, and colorless kernels were selected from homozygous marbled ears. Plants grown from these kernels were self-pollinated, and the progeny ears were evaluated for degree of spotting. Repetition of this procedure in successive generations resulted in the isolation of marbled sublines with distinctly different but still variable phenotypes. The families ranged from very light, in which five percent or less of the homozygous

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marbled seed was spotted, to very dark, in which nearly all the kernels were extensively pigmented (Figure 1).

To ensure a uniform genetic background, the various W22 marbled sublines were again mated to the standard W22 inbred strain. Pollen from plants belonging to the homozygous marbled families was placed on the silks of W22 R^rR^r individuals. The resulting $R^{mb}R^r$ offspring were pollinated by W22 r^gr^g (colorless aleurone, green plant), and the $R^{mb}R^{mb}r^g$ seed was used to evaluate the marbled phenotype.

Aleurone spotting was scored by matching random samples of seed from the central portions of ears against a set of standard kernels. The latter comprised seven specimens, selected in such a way that the sample populations were distributed among eight aleurone color classes. The classes ranged from class one, which was colorless, to class eight, which was almost completely pigmented. Classes between one and eight represent various degrees of marbling.

A test was made to determine whether M^{st}, a modifier of stippled aleurone, affects the expression of marbled also. According to ASHMAN (1960), the difference between light stippled and standard stippled is due to a modifier located about 5.7 crossover units distal to the R locus. R^{st} strains carry the modifier (M^{st}), whereas light stippled lines lack it, thereby representing the null condition (m^{st}) with respect to this locus. The standard W22 inbred line is homozygous m^{st}.

FIGURE 1.—The diversity of marbled phenotypes. The ears represent three different homozygous marbled sublines in the same W22 inbred background.

Several light and dark marbled families were pollinated with both light and standard stippled. The heterozygous $R^{st}R^{mb}$ plants were pollinated with W22 r^gr^g (m^{st}), and the resulting ears were examined for recombinants differing in grade of aleurone spotting.

Marbled mutates characteristically to germinally transmissible self-colored aleurone. The mutants occur either as single kernels with self-colored aleurone or, less frequently, as single kernels with marbled or colorless endosperm but colored scutellum. About one third of the former and all of the latter class of kernels represent germinal mutations to self-color.

Since preliminary observations had suggested that the frequency of self-colored mutants might be greater in dark than in light marbled lines, three very light families (1–5 percent pigmented kernels) and their dark relatives (80 percent or more kernels pigmented) were analysed separately with respect to mutation rate.

Homozygous $R^{mb}R^{mb}$ plants were pollinated with W22 r^rr^r (colorless aleurone, red seedling), and the putative mutants were selected from the resulting $R^{mb}r^r$ ears. Upon selfing the plants in question, presumed mutants were considered valid only if they segregated both the mutant and r^r phenotypes. Loss of supposed mutants through failure of the seed to germinate, or death of the plants, was compensated for by a proportionate adjustment of the total kernel number in computing the mutation rate.

The $R^{mb}r^r$ seed on the ears bearing the mutant kernels was used to evaluate the aleurone phenotype of each of the marbled lines by the procedure previously explained.

The mutation frequency for one of the sublines also was determined in heterozygotes with r^r. $R^{mb}r^r$ progeny from the mating $R^{mb}R^{mb}$ ♀ × W22 r^rr^r ♂ were pollinated with W22 r^gr^g and the presumptive mutants were selected for further testing.

EXPERIMENTAL RESULTS

Variations in aleurone spotting pattern in W22 marbled sublines: The average aleurone color scores of $R^{mb}R^{mb}r^g$ kernels from the mating R^rR^{mb} ♀ × W22 r^gr^g ♂ are assembled in Table 1. Each score represents a 50-kernel sample taken from the central portion of each ear. Family W189 was obtained by placing pollen of a medium marbled subline on W22 R^rR^r plants. Its light marbled sib is represented by family W201.

The data in Table 1 show that phenotypic differences between closely related marbled sublines tend to persist when these lines are again backcrossed to the W22 inbred strain. The two sib families are distinct with regard to aleurone phenotype.

Noneffect on marbled of a modifier of the stippled aleurone pattern: The $R^{mb}R^{st}$ heterozygotes from the matings $R^{mb}R^{mb}$ ♀ × $R^{st}R^{st}$ ♂ invariably yielded some light stippled progeny when pollinated with W22 r^gr^g. Thus both the light and dark marbled families tested must regularly have carried m^{st}. Moreover, the $R^{mb}M^{st}$ counterparts of the light stippled recombinants ($R^{st}m^{st}$) were indistinguishable

TABLE 1

Aleurone color scores of $R^{mb}R^{mb}r^{g}$ *kernels following matings of W22* $R^{r}R^{mb}$ ♀ × *W22* $r^{g}r^{g}$ ♂

Female parent	Average aleurone color score	Female parent	Average aleurone color score
W189– 1	1.82	W201– 1	0.48
– 2	2.31	– 2	0.14
– 3	5.16	– 3	0.87
– 4	3.78	– 4	0.22
– 5	3.50	– 5	0.74
– 6	4.40	– 7	0.06
– 9	1.26	– 8	0.16
–12	1.86	– 9	0.30
–13	3.42	–11	0.22
–14	3.16	–12	0.12
–16	3.44	–13	0.78
Mean	3.1		0.37

phenotypically from the $R^{mb}m^{st}$ segregates on the same ear. These results show that the stippled modifier is not responsible for the phenotypic differences between the various marbled sublines.

Mutation of marbled to self-colored aleurone: The frequencies of mutation to self-color of the R^{mb} alleles associated with different aleurone phenotypes are presented in Table 2. As can be seen from the table, the light marbled families failed to produce any self-color mutants among 24543 gametes tested. The average mutation frequency to self-color for the corresponding medium and dark marbled lines was 11.0 per 10^4 gametes. Thus marbled lines which exhibit extensive aleurone spotting also show relatively high frequencies of germinally transmissible mutations to self-color.

The mutation frequency for one of the sublines (W200) in heterozygotes with r^r is given in Table 3. The data show a marked increase in the mutation rate of R^{mb} when the particular marbled line was made heterozygous with r^r ($\chi^2 = 10.18$, $P < .01$,1 d.f.). This finding is the reverse of that reported for stippled, in which the frequency of R^{sc} (self-color) mutations is greater in homozygotes than in heterozygotes with r^r (ASHMAN 1960).

TABLE 2

Frequency of mutation of R^{mb} *to* R^{scm} *(self-color) in various W22* $R^{mb}R^{mb}$ *sublines*

Subline	Mean aleurone color score of marbled parent strain	No. of gametes tested	No. of R^{scm} mutations per 10^4 gametes
W186	0.22	4,576	0.0
W200	0.27	14,967	0.0
W229	0.31	5,000	0.0
W249	2.13	4,410	6.8
W188	2.60	5,774	8.6
W226	4.90	2,834	17.6

TABLE 3

Frequency of mutation to self-colored aleurone in a light marbled family when homozygous and heterozygous with r^r

Genotype	Number		Number of R^{scm} mutations per 10^4 gametes tested
	R^{mb} gametes tested	R^{scm} mutants	
$R^{mb}R^{mb}$	14,967	0	0.0
$R^{mb}r^r$	11,751	8	6.8

DISCUSSION

The main question raised by the present findings concerns the source of the genetic variability in the marbled pattern in W22 stocks. Following the initial outcross of marbled to the standard W22 stock, the marbled segregates were backcrossed four times to the foundation inbred line, prior to recovery of the R^{mb} factor by self-pollination. During this period, there was no selection for particular marbled phenotypes. Consequently, the W22 marbled line so obtained and the W22 inbred strain would be expected to be closely similar, except at and in the vicinity of the *R* locus. Nevertheless, the aleurone spotting density in the recovered W22 marbled stock subsequently was readily shifted by selection. Moreover, the resulting marbled sublines, although distinct with regard to aleurone phenotype, continue to exhibit considerable dispersion for aleurone color index (data of Table 1) when further backcrossed to the W22 inbred strain. Whether the striking diversity of ear phenotypes within the two sublines represents a continuance of genetic variation in the marbled allele itself is a question to which further experiments should provide an answer.

The data show also that an increase in the amount of aleurone spotting is accompanied by an increase in the rate of mutation to self-color. Conceivably, the formation of a self-colored sector in the aleurone and the production of a transmissible self-colored mutant reflect the same kind of event, namely, the mutation of a colorless allele to a colored form. Differences between the various marbled sublines might then reside in a modifying gene closely linked to the *R* locus. This modifier could alter the stability of the R^{mb} allele, or else could change the time during development, when mutations to self-color occur. It is necessary, on this hypothesis, to assume a high rate of mutability of the modifier postulated.

An alternative interpretation which excludes the participation of a modifying gene of the conventional kind is more likely. The observed diversity might be due to occurrence of the marbled allele itself in a number of states of unlike stability. Frequent shifts between the various states in germinal and somatic tissues would account for the persistent heterogeneity in R^{mb} stocks.

Changes of state of a locus have been related to the transposition of controllers (McCLINTOCK 1951, 1956; BRINK and NILAN 1952), to changes in state of controllers (McCLINTOCK 1951), and to alterations in the mode of association of such an element with its locus (BRINK 1958b). The R^{mb} allele, as shown earlier, mutates at different rates to R^{scm} in $R^{mb}R^{mb}$ and $R^{mb}r^r$ genotypes. While it could

be supposed that R^{scm} mutants arise through crossing over and that heterozygosity enhances the frequency of this process, such an assumption is clearly incompatible with the observation that germinal and endosperm mutants occur independently of each other. Instead, the increased incidence of germinal mutations in $R^{mb}r^{r}$ heterozygotes may represent a specific response on the part of the R^{mb} allele to the presence of a particular genetic element in the homologous r^{r}-carrying chromosome, or it may reflect an interaction between a component of the R^{mb} allele in $R^{mb}R^{mb}$ homozygotes. An operational model of the latter kind is afforded by the variegated pericarp allele in maize. WOOD and BRINK (1956) obtained cogent evidence in this case that the increased frequency of mutation of variegated to self-red pericarp in heterozygotes, as compared with homozygotes, was a function of dosage of Modulator, a transposable element which, when present at the *P* locus, but not elsewhere, suppressed the action of the gene for self-red pericarp.

The marbled allele, similarly, may comprise a gene for self-colored aleurone plus an inhibitor at the *R* locus. The occurrence of stable mutations to self-color is compatible with this idea. Proof is lacking, however, that such mutations and the heritable variations in the marbled allele itself involve shifts in position of a pigmentation inhibitor relative to the gene for self-color.

SUMMARY

The paramutagenic R^{mb} allele which, by itself, conditions spotted aleurone and green plant in maize fails to give a constant aleurone phenotype when incorporated in a highly uniform inbred strain of corn known as W22.

Attempts to modify the amount of aleurone spotting in W22 marbled ($R^{mb}R^{mb}$) plants by selection resulted in the recovery of various marbled sublines differing in degree of aleurone pigmentation. In addition, the rate of mutation to germinally transmissible self-colored (R^{scm}) aleurone was found to be much higher in heavily pigmented marbled sublines than in their lightly spotted and near-colorless relatives. Presumably the formation of a colored spot in the aleurone is due to mutation of a relatively unstable colorless allele to a stable colored form in an endosperm cell. When such a mutation occurs in a cell lineage that includes the egg, then the self-colored allele is transmitted to the offspring.

Since the diversity observed between marbled sublines has its basis at, or in the vicinity of, the *R* locus, it is suggested that the various marbled phenotypes are controlled by an allele which regularly varies between several states of unlike stability.

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